THE
PRINCIPLES AND PRACTICE
OF
BREWING

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THE
PRINCIPLES AND PRACTICE
OF
BREWING

BY
WALTER J. ^{John} SYKES, M.D., D.P.H., F.I.C.

THIRD EDITION, THOROUGHLY REVISED
BY THE AUTHOR
AND
ARTHUR R. LING, F.I.C.
LECTURER ON BREWING AT THE SIR JOHN CASS INSTITUTE

With Numerous Illustrations

LONDON
CHARLES GRIFFIN AND COMPANY, LIMITED
EXETER STREET, STRAND

1907

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TO
DR. EMIL CHRISTIAN HANSEN,
PRINCIPAL OF THE CARLSBERG LABORATORIUM,
COPENHAGEN,

THIS WORK IS DEDICATED
IN TOKEN OF
ADMIRATION, RESPECT, AND ESTEEM;
ALSO IN RECOGNITION OF THE IMMENSE SERVICE WHICH
HE HAS CONFERRED ON THE FERMENTATION INDUSTRIES
BY HIS RESEARCHES, MORE ESPECIALLY BY THOSE
WHICH LED TO THE INTRODUCTION INTO
ACTUAL PRACTICE OF PURE YEAST
CULTURES DERIVED FROM ONE
SINGLE CELL.

PREFACE TO THE FIRST EDITION

AMONG the achievements of the latter half of the present century, few have been fraught with more beneficial consequences than the practical application of the deductions of science to manufacturing operations. Nearly every important industry has passed from the empirical to the scientific stage. This revolution has not been effected at a leap, but has been of relatively slow growth. Much prejudice had to be overcome, and for a long time the intervention of science was regarded with considerable mistrust. Perhaps in no other industry was this the case more than in that of brewing. Now all this is changed; the benefits which science is able to confer are generally recognised and appreciated, and where science was formerly repulsed, she is now welcomed with opened arms. That this is true with regard to the fermentation industries, is shown by the number of Institutions which have arisen in different parts of the world, during the last ten years, with the object of investigating the laws which govern the processes and operations carried on in malting, brewing, distilling, &c. In our own country, which has been one of the most backward in this respect, we have seen the founding of the Institute of Brewing and other kindred Institutions, which, together with their conjoint Journal, pay considerable attention to the scientific aspect of brewing.

During this short period an enormous amount of experimental investigation has taken place, the records of which are scattered over a vast amount of literature. It is the object of the present work to bring together, within the limits of a volume of a handy size, the salient points of this mass of literature, and to present, as it were, a bird's eye view of the science and art of Brewing.

The two fundamental principles on which so much depends, viz., the action of diastase on starch, and the alcoholic fermentation caused by yeast, have been treated at considerable length, and as far as possible in historic sequence, so as to present these subjects to the reader in the most interesting form. In the Chemical portion of the book an attempt has been made to classify the substances used by brewers, and those appertaining to the science of Brewing, in a systematic form consonant with the arrangement generally adopted in modern works on organic chemistry. Chapters on the Physics of Brewing, such as heat and density, are also included. Considerable space has been allotted to a description of Elementary Biology, a subject of great importance

to the brewer, but one which has been strangely neglected in most works on Brewing. The general methods of bacteriological research are also given, and a chapter is devoted to instructions for the use of the microscope, without the aid of which instrument bacteriology and the culture of pure yeast would be an impossibility. The interpretation of the results of the analysis of the materials used in brewing has been given, but the analytical methods of testing them have been omitted, as they would have made the book too voluminous, and constitute in themselves a subject sufficiently large to form a separate treatise.

In the practical portion of the work, the general methods employed in Malting and Brewing are given, but it is evidently impossible to include all the minor details, since these differ in almost every brewery, and can only be learned by actual experience in the brewery itself. The author trusts that the work will be found not only useful to those who are commencing a brewing career, and for whom it is primarily intended, but also to older, practical men who may be desirous of increasing their knowledge of the scientific side of brewing.

With these objects in view, he has attempted to present each subject in as lucid and intelligible a manner as possible, often, it is to be feared, at the sacrifice of literary elegance. Numerous references are given throughout the work solely for the benefit of those who wish to enlarge their knowledge of any particular subject; in the great majority of cases these have been verified from the originals, and the remainder have invariably been taken from reliable sources.

The author would here express his indebtedness to many friends for much kind assistance in the compilation of the work, particularly to Mr. John Marriott and Mr. Harris S. Beaman, who have revised, respectively, the sections on Practical Malting and Practical Brewing; also to Mr. A. Ashe for kindly looking over the microscopical portion, and to Mr. Arthur R. Ling (Editor of the *Journal of the Federated Institutes of Brewing*), and Mr. Charles A. Mitchell for assistance in correcting the proofs. A few of the illustrations in the bacteriological portion are from blocks lent by Mr. C. Gerhardt, of the Chemical Instrument Repository, Bonn, and some of those of brewery utensils are supplied by Messrs. Llewelyn & James of Bristol.

WALTER J. SYKES, M.D.

CROYDON, August 1897.

PREFACE TO THE THIRD EDITION

THE Second (Revised) Edition of this book was published in December 1902, and received the same appreciative recognition as had been accorded to the original work. The steady sale quickly made it necessary to prepare for yet another edition, but Dr. Sykes, owing to a serious accident which he had met with in the summer of 1902, felt unequal to the task, and he accordingly sought my co-operation. The revision was completed and the present edition was practically through the press in December last when, to the profound regret of all, Dr. Sykes died.

Following the original object, the endeavour has been made, in bringing the work up to date, to add to the text a succinct but accurate account of researches on the subject which have been made during the last few years. It is believed that no important researches have been omitted.

Large additions have obviously been necessary notably in connection with stereochemistry, the chemistry of the carbohydrates and the enzymes, and more particularly in regard to the action of diastase on starch. The section dealing with hops has been considerably enlarged especially from the chemical point of view. Under vegetable biology and fermentation will be found numerous emendations and additions, and the chapters on barley, malting and brewing have been considerably extended. In order to include an account of certain researches published during the latter part of 1906 and the commencement of the present year, it has been found necessary to add an Appendix.

I have to express my indebtedness to the Council of the Institute of Brewing, the Editor of the *Brewers' Journal*, Messrs. R. J. Hanbury & Co., Messrs. S. H. Johnson & Co., and Messrs. Schmidt and Haensch for the use of blocks. I also desire to thank my assistant, Mr. T. Rendle, for help in connection with reading the proof-sheets.

ARTHUR R. LING.

THE LABORATORIES,
74 GREAT TOWER STREET,
LONDON, E.C.

June 1907.

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ERRATA

Page 33, the formula for formaldehyde should be =

Page 189, line 9 from bottom, *for* " or " *read* " and "

Page 191, line 11 from top, *for* "(138° F.) *read* "(158° F.) "

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A THEORETICAL AND PRACTICAL TREATISE ON BREWING

INTRODUCTION

THE principles involved in the processes of malting and brewing fall under the three scientific heads of Physics, Chemistry, and Biology.

To the first of these categories belong all such operations as the heating evaporation, and freezing of water, in which no chemical change is produced, but simply an alteration of its physical state ; also all those operations which are of a purely mechanical nature, such as the raising of water by pumps, the sorting and cleansing of barley, &c.

Under the second head are included all those processes in which a chemical change is brought about in some of the materials employed. To these the operation of mashing belongs, in which the starch of the malt is so profoundly modified that it no longer exists as such, but is metamorphosed by the agency of the unorganised ferment or enzyme diastase into sugar and other bodies that differ considerably in chemical composition from the original starch, and, consequently, possess entirely different properties.

To the third category belong those processes in which chemical changes are effected through the agency of life, either in organisms or by their aid. Of such a nature is the process of malting, in which, during the germination of the barley, profound chemical changes are brought about in the constituents of the barleycorn by vital agency. So, also, in the fermentation of wort, which is effected through the instrumentality of the living organism "yeast," whereby the sugar of the wort is converted into a series of bodies, alcohol, carbon dioxide, &c., entirely different in their properties from the original sugar.

The broad lines on which the operations of malting and brewing are based had been fixed by practical experience long before anything was known of the scientific principles underlying the methods employed, and a number of rules gradually came to be formulated, by the observance of which the brewer was enabled, although he might possess no scientific knowledge, to carry on his operations with a greater or less degree of success. But the brewer who worked under such conditions was little better than an animated machine ; he simply followed a certain routine, and knew nothing whatever of the why

and wherefore of the various processes going on around him. So long as circumstances were favourable, and everything went on smoothly, he obtained good results ; but when some trifling irregularity occurred in the manipulation of the highly sensitive materials he employed, then the whole brewing process became disorganised, often with the effect of entailing serious pecuniary losses. In such a case the non-scientific brewer is helpless. As Bersch says, he is just as unable to detect the cause of his trouble, with a view to its remedy, as an ordinary individual is to locate the fault in his watch when it has stopped from some hidden cause. His attempts to bring matters into their regular groove are merely the outcome of so much conjecture ; he may by chance hit upon the right thing to do under the circumstances, or his endeavours may make matters still worse. Fortunately, this state of affairs is now almost a thing of the past ; there is hardly to be found nowadays a brewer who does not possess more or less knowledge of the principles involved in the daily routine of his work.

It is the province of science to afford an adequate explanation of the principles involved in those chemical and biological changes which take place in the materials dealt with in the process of brewing, and just in proportion as these principles become understood and practically applied, so is precision and certainty introduced into actual working. The brewer who possesses an intimate acquaintance with the principles governing his operations is able to make allowances for variations in his materials and alterations in his working conditions ; he is therefore in an infinitely better position to secure uniformly good results than one who is only able to work by rule of thumb.

But the theoretical explanation of the phenomena involved in some of the processes employed in the manufacture of malt and beer lead into the most recondite, and, at times, into quite unexplored territories of scientific inquiry ; hence, where this is the case, an adequate scientific explanation of the observed phenomena is as yet unattainable ; consequently, brewing still remains to some extent an empirical art. But as science, which is now making rapid strides, progresses, so will the remnant of empiricism which still remains disappear from the brewery, and real definite scientific knowledge, with its consequent advantages, take its place.

As brewing is a commercial undertaking, the brewer who wishes to be a master of his business must possess, in addition to much scientific knowledge, a commercial training. Like every other workman, he has to handle tools, and thorough efficiency in the use of those appliances in the brewery which in reality are his tools can only be obtained by actual experience in the brewery and malthouse, hence the value of an apprenticeship. It is only the harmonious blending of theory with practice that will enable a brewer to carry on his operations with that enhanced degree of certainty and with that economy which are so requisite in these days of keen competition.

PART I

CHAPTER I

PHYSICAL PRINCIPLES INVOLVED IN THE PROCESS OF BREWING

HEAT

THE brewer, in common with every other manufacturer, is largely concerned with the application and adaptation of the forces of Nature to his requirements. Many of the processes in the operation of brewing, such as boiling, evaporating, cooling, &c., are effected through the agency of heat.

Definition of Heat.—Heat may be defined as that agent which, when applied to the human body, produces the sensation of “warmth.” It is that form of energy which, when communicated to a substance, produces what we term temperature. The tendency of all bodies, when in contact with one another, is to assume a state of equilibrium as regards heat, and when this state of equilibrium has been attained, they are said to be of the same temperature. Thus, when we touch a body which is hotter than the temperature of our own bodies, it gives off heat to our hand, and consequently we feel the sensation of warmth; conversely, if the body touched be colder than ourselves, some of the heat of our hand passes to it, and we perceive the sensation of cold, which is really the feeling caused by heat passing away from our body. It must not be supposed that any actual substance is transferred in either of these cases, for heat is not a material substance, but a state of movement in the imponderable ether which is assumed to pervade all substances and all space. It is, however, exceedingly convenient to speak of heat as if it were a material substance passing from one body to another, and this conduces much to simplicity in describing the phenomena of heat.

Conduction of Heat.—If, while holding the end of a rod of copper, we place the other end in the flame of a spirit-lamp or other source of heat, we find that the whole rod speedily becomes so hot that we can no longer hold it. If, however, we try a similar experiment with a rod of glass, we find that the end which is opposite to that in the flame never becomes perceptibly hot, however long we may hold it. The reason for this is, that copper and the other metals belong to that class of bodies which allow heat to pass

along them readily, and are hence called "good conductors;" whilst glass belongs to the opposite class, called "bad conductors," since they only allow heat to pass along them with extreme difficulty. These two properties of bodies are very frequently made use of in the construction of brewing utensils; for instance, when the brewer wishes to prevent as much as possible the loss of heat from his steam-pipes, or from his mash-tun, if it be a metal one, he clothes these portions of his apparatus with badly conducting materials, such as asbestos, or a mixture of cork and clay, &c. When, on the other hand, he wants the heat to pass rapidly from his worts to his cooling liquor, he interposes between the two fluids in his refrigerator a thin sheet of copper, which is an excellent conductor of heat.

Convection of Heat.—Liquids as a rule are exceedingly bad conductors of heat. If, for instance, a lump of ice be put in the bottom of a test-tube, a small coin placed over it to retain it in position, the test-tube partially filled with water, and the flame of a lamp applied to the tube just beneath the surface of the liquid, its upper layer may be boiled for some considerable length of time without melting the ice. As is well known, the whole of the water or other liquid contained in a vessel may be readily heated, provided the flame or other source of heat be applied to the bottom of the vessel; but here we have to do with another method of conveying heat—viz., convection. This method may be ocularly demonstrated by taking a large glass flask and nearly filling it with water, adding a few pinches of powdered cochineal or some other body of about the same specific gravity as water, and placing beneath the flask a lamp or other source of heat. It will be seen that a current is soon established, which ascends through the centre of the fluid, and, on arriving at its surface, bends over in every direction like the foliage of a palm-tree, and forms a number of descending currents. These travel downwards in proximity with the sides of the flask until they reach its heated portion, when they again ascend in the hot central current; thus, in its turn, every portion of the water becomes exposed to the action of the heat. The reason this occurs is that the water when heated expands and becomes specifically lighter than cold water; consequently it ascends, whilst the colder portions simultaneously descend to replace the portions which have ascended, and this action goes on continuously until the temperature at which water boils is reached. Hence, in the process of heating by convection, the force of gravity also comes into play.

In cooling, the reverse process takes place; the portion of water in contact with the air, or with some substance colder than itself, loses its heat, contracts, and becomes heavier; consequently it sinks. The warmer portions meanwhile ascend, and, after being cooled, descend; this goes on continuously until the whole of the fluid arrives at the same temperature as that of the cooling medium. Hence, any apparatus for cooling a mass of liquid, such as the attemperor in a fermenting vat, should be placed near the surface of the liquid, or the portion of the latter above it will not receive its due share of the cooling effect of the apparatus.

Gases are also bad conductors of heat; they likewise are heated by convection. A familiar instance of this is the draught produced by the fire in an ordinary chimney. The air heated by the fire expands, becomes specifically lighter, and consequently rises, forming a current which is rendered visible by the particles of carbon carried along with it. Currents of colder air continually descend from the ceiling and sides of the room to take the place of the heated air which has passed away. In order therefore to heat the air in a room equally, the source of heat should be placed as near the floor as possible. Conversely, when it is sought to cool the air of a room, the agency for the withdrawal of heat is placed as near the roof as possible; thus the refrigerating pipes occasionally employed for keeping down the temperature of a fermenting-room or storage-cellar are placed near the ceiling.

Radiation of Heat.—There is yet another method by which heat is propagated, viz., radiation. Every hot body, in addition to losing heat by convection and conduction, also loses heat by radiation. This may be shown by hanging a hot body in a vacuum, when it is found to rapidly grow colder, though the loss in this case cannot be due either to conduction or convection. Heat propagated by radiation moves in straight lines, precisely in the same way as light does; indeed, both light and heat are caused by vibrations of the ether, which are exactly similar in all respects except that of wave length. Thus a body, on being gradually heated, at first only emits heat rays: as it gradually grows hotter it begins to emit rays of light as well, and the hotter it becomes the greater the number of light rays it sends forth. A familiar illustration of this is seen in the glowing carbon filament of an incandescent electric lamp, which, being intensely heated by the current of electricity passing through it, emits a large number of light rays.

The rate at which heat passes away from a hot body by radiation is much influenced by the state of its surface; a bright polished surface radiates heat with comparative slowness, whilst a dead black surface radiates heat with great rapidity. Conversely, a body which radiates heat readily also absorbs it rapidly, and a body which parts with its heat by radiation slowly also absorbs it slowly. Hence the cover of a mash-tun, when made of copper, should be kept as bright as possible, in order to prevent loss of heat by radiation. Steam-pipes, and all the vessels in the brewery from which it is sought to prevent loss of heat by radiation, are covered with some non-conducting material.

Expansion by Heat.—Almost all bodies expand when heated. Of the various classes of bodies, gases expand the most, solids the least, and liquids occupy an intermediate position. Each body has a degree of expansibility under the influence of heat peculiar to itself; thus, amongst metals, zinc expands the most, and probably platinum the least. In the case of liquids, those which are most volatile as a rule expand most. Alcohol, for instance, is more expansible by heat than water, and water more than mercury. All gases, on the other hand, have the same degree of expansibility for the same amount of heat or so nearly so that the exceedingly small deviation which

under certain circumstances they make from this rule need only be taken into account in the most delicate researches.

The Thermometer.—In many operations connected with brewing it is of the highest importance to have some convenient means for ascertaining correctly the temperature of the various substances we have to deal with, such as that of the malt on the growing floor, or on the drying kiln, the temperature of the worts at various stages of the process of brewing, that of the air in the fermenting-rooms, storage cellars, &c. This is fulfilled in an eminent degree by the “thermometer,” an instrument which is constructed in the following manner:—A glass tube of very fine and even bore is taken, and a cylindrical or spherical bulb is blown at one end of it. The bulb is then heated to drive out a portion of the air which it contains, the open end of the tube dipped into a vessel containing mercury, and the bulb allowed to cool. As the bulb cools, the contained air contracts, and a portion of mercury is drawn into it. This is then boiled, in order that its vapour may drive out and replace the remainder of the air and any moisture left in the bulb and tube. While the whole is hot, and the mercurial vapour is escaping freely from the open end of the tube, this end is placed under the surface of hot mercury, and the whole allowed to gradually cool down. When completely cold, the whole of the bulb and tube will be filled with mercury. The bulb is now gently heated, and a portion of the mercury driven out, the end of the tube being sealed up with the blow-pipe while the column of mercury still fills the tube. As the mercury contracts, a vacuum is left behind; if the tube were sealed while full of air, it would burst when the mercury again expanded. The tube ought then to be calibrated, in order to ascertain what differences there are in the diameter of its bore. For this purpose a small column of mercury about a third of an inch is detached from the main body of the fluid, moved up and down the tube, and its length noted in the various portions of the tube by the aid of a microscope. It is obvious that where the tube is narrower the detached column will be longer than in the wider portions; these differences are noted, and allowances made for them when the tube is subsequently graduated. It is now necessary to find two fixed points of temperature, and for this purpose the melting-point of ice and the boiling-point of water are chosen as being those most readily attainable. In order to find the position of the point in the mercurial column which corresponds to the freezing-point of water, the thermometer is placed in pounded ice in a melting state, care being taken that the water produced by the thawing of the ice has a ready means of passing away. When the column has assumed a stationary position, a small mark is made on the tube indicating its height. The next operation is to subject the thermometer to the action of steam evolved from water boiling at the pressure of the atmosphere, and this is effected by placing it in a long, upright, cylindrical vessel, in the bottom of which water is kept boiling. An aperture is provided at the side of the vessel for the free escape of steam, so that the pressure inside the vessel may not rise beyond that of the external atmosphere. When, after

a little time, the end of the column has become stationary, another mark is made on the tube to indicate the temperature of boiling water. This is not an absolutely fixed point; water boils at different temperatures, according as the pressure to which it is subjected varies. Thus, when the air has a temperature of 32° F., and the barometer indicates a pressure equal to 29.315 inches of mercury, water boils at 211° F.; when it indicates a pressure of 30.444 inches, the air being at 32° F., water boils at 212.9° F. In order that this variation may not give rise to practical difficulties, the boiling-point of water (212° F.) has been fixed by a Government Commission, who have determined that the temperature at which distilled water boils under a pressure of 29.905 inches of mercury, with air at 32° F., shall be indicated by 212° F. Consequently, when the position for this point is ascertained, with the air at any other pressure and temperature than this, a corresponding correction must be made. These two fixed points having been ascertained, the space between them is divided into degrees; in England it is customary to divide it into 180 degrees, as proposed by Fahrenheit; in France it is divided into 100 degrees, on the plan introduced by Celsius; so that 0° indicates the freezing-point of water and 100° its boiling-point, hence this is called the "Centigrade scale." This latter is by far the most rational method of division, and consequently is the one almost exclusively used by scientific workers throughout the world. There is yet another method, that of Reaumur, which is much used in the breweries of Germany and Austria; in this the space between the freezing and boiling points of water is divided into 80 degrees. By whatever method the thermometer is graduated, degrees of the same size as those between the two ascertained fixed points are marked off above and below these points, always taking into consideration the necessary corrections for the calibre of the tube. Other liquids besides mercury are sometimes used for filling thermometers, such as alcohol, carbon disulphide, &c. But though alcohol has the advantage of not freezing at ordinary low temperatures (mercury freezes at -39° C., -38° F.), it has the disadvantage of boiling at the comparatively low temperature of 78.39° C. (173.1° F.), so that a thermometer filled with alcohol would be entirely unfitted for many purposes in the brewery. Another reason which leads to the choice of mercury for this purpose is that this liquid expands in equal increments for each degree of heat between the freezing and boiling points of water, a property not shared by most other liquids or solids.

Conversion of the Readings of the Various Thermometric Scales into each other.—To reduce degrees Centigrade to degrees Fahrenheit the following formula may be employed :

$$C.^{\circ} \times \frac{180}{100} + 32 = F.^{\circ}; 60^{\circ} C. \text{ would therefore be } 60 \times \frac{9}{5} + 32 = 140^{\circ} F.$$

To convert degrees Fahrenheit into degrees Centigrade :

$$(F.^{\circ} - 32) \times \frac{5}{9} = C.^{\circ}; 140^{\circ} F. \text{ would be } (140 - 32) \times \frac{5}{9} = 60^{\circ} C.$$

Similarly, degrees Reaumur to degrees Fahrenheit and the converse are effected by the following formulæ :

$$R.^{\circ} \times \frac{9}{4} + 32 = F.^{\circ}$$

$$(F.^{\circ} - 32) \times \frac{4}{9} = R.^{\circ}$$

Sources of Error in the Thermometer.—The bulb of an ordinary glass thermometer gradually contracts to a slight extent for some time after it is made ; consequently, if graduated immediately after it is filled, its readings after a time become too high. This error may amount to $+ 1^{\circ} C.$ ($1.8^{\circ} F.$), which is about the extreme limit of the change. Consequently, all thermometers should be allowed to rest for some considerable length of time after being filled before they are graduated. All thermometers used for delicate researches are now made of the so-called Jena glass, which contains, in addition to the ordinary constituents of glass, a small quantity of zinc. When a thermometer tube made of this glass, is exposed to a temperature of $300^{\circ} C.$ for thirty hours, after subsequent filling and graduation its bulb does not contract on use, or at most to an almost imperceptible extent. Consequently, it is advisable to have in every brewery a standard thermometer of this kind, so that the rest of the thermometers can be tested against it for correctness from time to time. A thermometer which has been graduated in an upright position should always be used in the same position, since the hydrostatic pressure of the column of mercury tends to enlarge to a slight extent the capacity of the bulb, and as the capacity of the bore is very small relatively to that of the bulb, small alterations in the latter are enormously magnified in the reading of the column. If a thermometer be suddenly heated and then cooled, as, for instance, if it be heated to the temperature of boiling water and then suddenly plunged into ice, its freezing-point will have altered slightly, and may read $0.1^{\circ} C.$ higher, and only in ten days or a fortnight will it have returned to its original reading. In taking readings with a thermometer, the instrument should always be immersed in the liquid or other substance which it is desired to take the temperature of as far as the length of the column of mercury extends. This is always carefully done when the two fixed points of the instrument are ascertained for the purpose of graduation. For instance, if a thermometer has its bulb only immersed in boiling water, the stem and the column of mercury it contains will only have the temperature of the air which surrounds them, and obviously the column will be shorter than if it were at the temperature of boiling water, hence the reading will be slightly too low. The tube being also cold, contraction will diminish its bore to a slight extent, and this, by lengthening the column of mercury, will tend to rectify the error to a small extent. But if an attempt be made to ascertain the temperature of any liquid approaching that of boiling water in this way, the error in the reading of the thermometer may be from 2° to $3^{\circ} F.$ too low.

As many of the thermometers met with in commerce, owing to careless graduation, have the freezing-point several degrees out of position, all these instruments, before being taken into actual use, should be carefully tested

against a normal thermometer; and this should be repeated every few months, in order to ascertain that their indications remain correct.

Thermometers are of various forms. For instance, the form used for ascertaining the temperature of the mash-tun is of considerable length, and is enclosed in a wooden or metallic tube, a sliding door or other suitable arrangement being provided to cover and protect the bulb when not in use, whilst the uppermost portion where the readings are taken is also protected by a small door.

In taking the temperature of a body with the thermometer, it is necessary that the instrument should come into as intimate contact with it as possible. When the body is gaseous this presents no difficulty. In taking the temperature of solid or liquid bodies, such as malt, water, wort, &c., care must be taken not only that the bulb, but also that the whole of the mercurial column is immersed in the substance, only so much of the column being left exposed as is just necessary to obtain a reading.

In taking the temperature of a mash considerable time must be allowed to elapse, as the heat has to penetrate through a considerable amount of material before the tube and mercurial column reach the temperature of the mash. It is also advisable to take the temperature of the mash in several places, then to add all the various readings together, and divide their sum by the number of readings taken. In this way we obtain an average reading.

When taking the temperature of malt on the drying kiln, care must be taken that its bulb does not come in contact with the floor, or too high a reading will be obtained. In order to obviate this, thermometers made for the drying kiln have a peculiarly shaped stand, which prevents the bulb coming in contact with the tiles.

An instrument called a "thermograph" has been devised for obtaining a continuous record of the temperature of the malt when on the floor of the drying kiln. It contains a cylinder, driven by clockwork, revolving once in twenty-four hours, to which a sheet of ruled paper is attached. On this the record is marked by a pen attached to a peculiarly constructed thermometer.

Unit of Heat.—It must be remembered that the thermometer only measures the *intensity* of heat; it does not measure its *quantity*. In order to measure the quantity of heat, two other factors must be taken into account—the *specific heat* of the substance in question and its *weight*. It is a matter of great convenience to have some unit for heat, just as we have for weights and measures. The quantity of heat usually taken for this unit is that required to raise one kilogramme of water from 0° to 1° C., but any other quantity of water and degree of heat on any other scale may be taken; consequently, as a matter of convenience, we shall occasionally take for the unit the quantity of heat required to raise one pound of water from 32° to 33° F.

Specific Heat, or Heat Capacity.—Bodies, when indicating the same degree of sensible temperature, differ very considerably in the amount of heat

which an equal weight of them contains. If two pieces of metal of the same size, shape, and weight—the one of silver and the other of copper—are suspended by means of pieces of thin wire in a vessel of boiling water for a few minutes, until they have attained the temperature of the water, and are then withdrawn and suspended in the air, their temperature will gradually fall; the piece of silver, however, will be found to lose its heat much more rapidly than the piece of copper. Hence we may conclude from this that an equal weight of copper at the temperature of boiling water contains much more heat than an equal weight of silver. As the copper parts with much more heat than the silver does in cooling, it must have required much more heat to raise it to the temperature of boiling water. Consequently, we say that the specific heat, or the heat capacity, of copper is greater than that of silver. Taking water as unity the specific heat of any other body is the quantity of heat which is necessary to raise the temperature of one kilogramme of it from 0° to 1° C°. It has been found by properly conducted experiments that, on this standard, the specific heat of copper is 0.095, and that of silver 0.057.¹

When equal weights of different substances, having different temperatures, are mixed together, the temperature of the mixture can only be the mean of those of the constituents, providing these have the same specific heat. Mercury has a much less specific heat than water; consequently, if a pound of mercury having a temperature of 100° C. be shaken up with a pound of water at 0° C., the resulting temperature of the mixture will not be the mean, 50° C. (as would have been the case if a pound of water at 100° C. had been mixed with the same weight of water at 0° C.), but will be found to be 3.2° C. The mercury has, therefore, lost 96.8° C. of heat to raise the temperature of an equal weight of water 3.2° C. only. Conversely, if a pound of water at 100° C. be shaken up with a pound of mercury at 0° C., the temperature of the mixture will be 96.8° C. Thus one unit of heat would raise a pound of mercury $\frac{96.8}{3.2} = 30.25^{\circ}$ C., consequently $\frac{1}{30.25}$ (or 0.033 of a unit of heat would raise that quantity of mercury one degree); 0.033 is therefore the specific heat of mercury.

Specific Heat of Malt.—In most works on brewing the specific heat of malt is stated to be rather less than half that of water, viz., 0.42. For reasons already stated it is highly necessary to take this into consideration when

¹ A relation between the specific heats and the atomic weights of the elements was shown to exist by Dulong and Petit in 1819, the product of the specific heat of an element multiplied by its atomic weight being nearly a constant quantity, thus:

	Specific Heat.	Atomic Weight.	Product.
Copper . .	0.0951	63.5	6.0389
Silver . .	0.0570	108.0	6.1560
Magnesium .	0.2499	24.0	5.9976

The numbers in the last column are known as the atomic heats of the respective elements. Compound bodies also have molecular heats, but these vary with the class of compounds; thus with the chlorides of the metals it is about 19, with their carbonates about 21 to 22.

calculating what the initial heat of a mash is to be. Within recent years Brown and Morris¹ have pointed out on the evidence of carefully conducted practical experiments that the specific heat of *dry* malt is 0.52. They showed, moreover, that when ground malt and water, both of which have attained the surrounding temperature, are mixed together, and the mixture stirred in the presence of a delicate thermometer, provided the malt is reasonably dry, a rapid rise of temperature, amounting to several degrees Fahrenheit, invariably takes place. This they attribute to the heat of combination of the malt and water. Under the name of "heat of imbibition" this phenomenon has long been known to botanists, and although it is more strongly marked in starch than in any other substance, brewers' grains, after drying, exhibit it to the same extent as malt itself. Brown and Morris were unable to obtain reliable results in the direct determination of the specific heat of malt, owing to the nature of the material and the imperfect apparatus at their disposal. In the practical experiments before alluded to, however, the temperatures of the grist and of the water, the quantities of the grist and of the water, the percentage of water in the grist, and the initial temperatures were all carefully taken. When these results were worked out, making proper allowance for the heat of hydration, it was invariably found that the calculated initial came out several degrees higher than the observed initial, when the specific heat of malt was taken as 0.42. The specific heat of dry malt was calculated from the whole of the experiments, and the value which agreed best with the results was 0.52. Absolutely dry malt is, of course, not met with in practice, but the specific heat of any brewery malt can be found by adding to the value just given for dry malt 0.005 for each per cent. of water it contains. Brown and Morris suggest that the hitherto accepted value for the specific heat of malt, 0.42, may have been arrived at from actual brewing operations, but that the heat of hydration was probably ignored in the calculations. They regard it as probable that the number was obtained from the first stages of a Continental decoction mash, in which the liquor rate is high; it would only require the malt to contain about five per cent. of moisture—by no means an improbable quantity—to make the specific heat work out at 0.42, when the heat of hydration is neglected.

When therefore the percentage of moisture in a malt is known, the "initial" which will be given by mashing that malt with a certain striking heat can be calculated with a high degree of accuracy, and conversely the striking heat which will be necessary to produce a given initial heat.

Brown and Morris determined the heat of hydration of flaked maize, and found it to agree with that of malt. The foregoing conclusions can therefore be applied to a grist containing a proportion of flaked material, and presumably grits and other forms of raw grain.

The table constructed by Brown and Morris showing the temperatures resulting from mixing malt and water of different temperatures is given in the practical portion of this volume under "Mashing."

¹ *Journal Fed. Inst. Brewing*, 1899, 5, 338.

Latent Heat of Solidification.—All solids, when they pass into the liquid state, absorb a certain amount of heat which can no longer be detected by the indications of the thermometer; in other words, a certain amount of heat has passed from the sensible into the latent state. If 1 lb. of snow or pounded ice be added to 1 lb. of water at a temperature of 79°C ., when all the snow or ice is melted the resulting 2 lb. of water will be found to have a temperature 0°C .; whereas, if 1 lb. of water at 79°C . had been added to 1 lb. of water at 0°C ., the result would have been 2 lb. of water at 39.5°C ., or the mean between 79°C . and 0°C . In melting, the 1 lb. of ice has absorbed 79°C . of heat, of which the thermometer now gives no indication; hence 79°C . have disappeared, or, in other words, become latent; they have been used in converting the ice from the solid to the liquid state. All other solids behave in a similar manner in passing from the solid to the liquid state; they render latent a certain amount of heat, and this is retained so long as the substance remains liquid.

When a liquid passes from the liquid to the solid state, its latent heat is evolved and becomes again sensible. It is difficult to illustrate this with ice, but the phenomenon may be readily shown in another manner. If to water boiling in a flask sodium sulphate be added until no more is dissolved, the flask rapidly corked while steam is still issuing from it, and the whole allowed to remain undisturbed until it is quite cold, the whole of the salt remains in solution, and what is known as a super-saturated solution of sodium sulphate is obtained. This is a solution which contains much more of the salt than cold water under ordinary circumstances would dissolve; for boiling water is able to dissolve 42.5 per cent. of this salt, whilst water at freezing-point can only take up 5 per cent. If the flask be now carefully uncorked, and the surface of the solution touched with a rough solid body, or a crystal of sodium sulphate dropped in, the whole mass instantly solidifies. That this conversion of the liquid into the solid state is accompanied by the evolution of heat may be readily perceived on grasping the flask with the hand; or, if a thermometer be introduced into the mass while still liquid, as solidification takes place the temperature will rise $18\text{--}20^{\circ}\text{C}$.

Latent Heat of Vaporisation.—When a liquid is converted into vapour, much heat is also rendered latent. This is markedly the case with water. If a vessel containing water at 0°C . be placed over a steady source of heat, and the length of time noted which is required to raise its temperature to 100°C ., which, say, is ten minutes, it will be found that a period of time about five times this length, or about fifty minutes, will have elapsed when all the water has evaporated away. Since the water received 100° of heat during the first ten minutes, it must have received approximately 500° during the fifty minutes. But we know that the temperature of boiling water at the pressure of the atmosphere never rises beyond a certain point, and that a thermometer introduced into the steam from boiling water indicates the same temperature as the water itself. Consequently, water in passing from the liquid to the gaseous state renders latent about four times

as much heat as is necessary to raise it from 0° to 100° C. This may be also shown in the converse manner. If 537 grammes of water be placed in a tared beaker, which is swathed in cotton wool or some other bad conducting material so as to prevent loss of heat by radiation, a thermometer inserted into the water, and steam allowed to bubble through the liquid, the beaker and its contents being removed and weighed immediately the thermometer indicates a temperature of 100° C., the water will be found just to have increased in weight by 100 grammes. This shows that the amount of heat contained in the steam of 100 grammes of water is able to raise the temperature of 537 grammes of water from 0° to 100° C. In addition to the 100° of sensible heat, steam therefore contains 437° of latent heat.

Theoretically, if no loss of heat took place by radiation, &c., the steam evolved from 100 gallons of water in the steam boiler would be able to bring to the boiling-point 437 gallons of water in the hot-liquor back, and yield in this way, taking into account the 100 gallons of water produced by the condensed steam, 537 gallons of water at boiling-point.

Evaporation.—By this is understood the process in which a liquid quietly and silently passes into the state of vapour. The rapidity of the process depends upon several factors. The higher the temperature of the liquid, the more rapidly it evaporates. The larger the amount of surface exposed to the air, the greater the amount of evaporation. Thus a cooler is so constructed that the wort on it shall present a large exposed surface to the atmosphere, to increase the evaporation as much as possible; and as a large amount of heat is absorbed by water in passing from the liquid to the gaseous state, rapid evaporation means rapid cooling. Evaporation goes on much more rapidly in dry air than in air which is considerably charged with moisture; hence the comparative slowness with which cooling proceeds on a foggy day, when the atmosphere is saturated with moisture. The constant removal of those portions of air in immediate contact with the surface of the liquid which have become highly charged with aqueous vapour, and the substitution of dry air for these, also considerably increases evaporation. Hence coolers are invariably placed in a room, the windows of which are provided with louver-boards for the admission of a current of air; and the process is often accelerated by placing a fan driven by power in the centre of the cooler, which causes rapid currents of air to traverse the surface of the liquid.

Evaporation goes on with extreme rapidity in a vacuum, so quickly that it is possible to freeze water by its own evaporation, if an arrangement be made to remove the aqueous vapour as fast as it is formed. This is generally accomplished by taking a flat vessel containing sulphuric acid, a substance which has a great attraction for water, and placing over it a small metallic capsule containing the water to be frozen. These are placed under the bell-glass of an air-pump and the air exhausted, when evaporation takes place so rapidly that the temperature of the water is speedily brought down to its freezing-point. This principle of reducing the heat of a liquid and of those bodies

with which it is in contact by rapid evaporation receives practical application in the action of freezing machines. In these, liquids which have a much lower boiling-point than water are generally employed, and they, when caused to rapidly evaporate by the partial vacuum maintained in the machine by its pump, absorb an enormous amount of heat. The following table gives the boiling-points of some of the liquids commonly used in freezing machines :

	Boiling-points in Degr. C.	In Degr. F.
Ether	+ 35.5	+ 96.0
Sulphurous anhydride	- 10.0	- 14.0
Ammonia	- 33.0	- 27.4
Carbonic anhydride	- 78.2	- 108.8

DENSITY AND SPECIFIC GRAVITY

One of the most frequent operations of a purely physical nature performed in the brewery is the determination of the specific gravity of wort, beer, and other liquids. The following is a brief description of the principles upon which this simple operation is based.

Density.—If we compare the relative proportions in size of a pound of lead and a pound of cork, we see at once that equal weights of the two bodies occupy very different volumes or spaces. Similarly, if we have a piece of cork and a piece of lead of the same size, the two bodies will have very different weights. Expressing these facts in more scientific language, we say that the densities of these two substances differ considerably; that lead possesses a much higher density than the cork. In familiar conversation we often express the same idea when we say that a substance, such as lead, is “heavy;” that another substance, such as cork, is “light.” Similarly, liquids have very different densities; thus a litre of water weighs 1000 grammes, a litre of mercury weighs 13,600 grammes, a litre of alcohol weighs 790 grammes. In order to express the density of a body, the simplest method is to weigh a known volume of it, and to divide the weight thus obtained by the volume; in this way the weight per unit volume is arrived at. Density, therefore, is only a relative term; we may say that the density of alcohol is $\frac{790}{13,600}$ that

of mercury, or that the density of water is $\frac{1000}{13,600}$ that of mercury. But

if a list of the varying densities of bodies is to be compiled, it is obviously necessary that the weight of a volume of each of them should be compared with the weight of an equal volume of some other body which shall serve as the standard, and that this latter substance should also be one which is readily attainable. In the case of solid and liquid bodies, water is chosen for this purpose; in that of gases, air—since these two substances are always at hand.

Specific Gravity.—The term “specific gravity of a body” expresses the ratio of its density to that of the substance chosen as the standard, that is—in the case of solids and liquids—water; or, in other words, the ratio between the weight of a given volume of the body under investigation and that of a similar volume of water. As the densities of bodies vary considerably with their temperatures, when the specific gravity of a substance has to be determined, not only must its own temperature be taken into account, but also that of the body with which it is to be compared. In taking the specific gravities of liquid or solid bodies a temperature of 4° C. (39.2° F.) is often chosen, this being the temperature at which water is at its maximum density: water, when cooled from 4° to 0° C. (32° F.), expands. Since at some seasons of the year it is exceedingly inconvenient to reduce bodies to such a low temperature, in ordinary practice a more readily attainable temperature is chosen, and this is 15.5° C. (60° F.). The specific gravity of a gaseous substance is generally determined at the temperature of the air at the time the operation is performed, and the volume it would occupy at any other temperature found by calculation.

Methods of obtaining the Specific Gravities of Solids.—In ascertaining the specific gravity of a solid it would be almost impossible, in most cases, to obtain a bulk of water equal to that of the solid. Advantage is therefore taken of the principle, first enunciated by Archimedes and applied by him to this particular purpose, that when a solid is immersed in water it loses exactly as much weight as that of a volume of water equal to its own bulk. In actual practice, the specific gravity of a body is found by weighing the substance first in air, then in water of a given temperature. This is accomplished by having a scale-pan attached to the balance, with shorter attachments to the beam than the ordinary pan has. On the under side of the former a hook is fixed, to which the body to be weighed in water is attached by a piece of thin string or wire. The weight of the body is first taken in air, and then a vessel containing water is placed under the scale-pan, and the body again weighed immersed in water. These two weights furnish the required data. Thus, supposing a piece of lead weighs in air 100 grammes, and when weighed in water it weighs 91.111 grammes; by subtracting 91.111 from 100 we obtain 8.889, and this is the weight of a volume of water equal to that of the lead. A simple proportion sum then gives the specific gravity of the lead:

8.889	:	100	::	1	:	11.25
Weight of equal vol. of water		Weight of substance in air		Specific gravity of water		Specific gravity of lead

In the case where the specific gravity of a substance which is soluble in water has to be taken, as, for instance, that of a crystal of sugar, it is first weighed in air, then in some liquid in which it is insoluble, such as oil of turpentine. The specific gravity of the oil of turpentine, or other liquid used for this purpose, is also ascertained, when a simple calculation gives the specific gravity of the body in question.

Specific Gravity of Gases.—This is carried out in a very simple manner. A glass globe provided with a stop-cock is weighed full of air; the air is then exhausted as completely as possible by an air-pump, and the weight of the empty globe taken. This will be less than the first weight, and this loss is the weight of the air which the globe contained at the temperature and the pressure of the atmosphere at the time the experiment was made. The globe is now filled with the gas to be examined, its weight again taken, due attention being being paid to the temperature of the gas and the pressure to which it is subjected. If these latter are the same as when the globe filled with air was weighed, then the following simple proportion sum gives the specific gravity of the gas, taking air as the standard :

$$\text{Weight of air} : \text{weight of gas} :: 1 : \text{specific gravity of gas.}$$

If the gas be weighed at a different temperature or at a different pressure, then these varying conditions will have to be taken into account and the requisite calculation made.

Methods of taking the Specific Gravities of Liquids.—This is by far the most important branch of the subject, so far as the brewer is concerned, since, in his numerous operations, he is very often guided by the varying specific gravities of the liquids with which he is constantly dealing. There are several methods of taking the specific gravity of a liquid, some of which leave nothing to be desired in the matter of exactitude.

FIG. 1.—Specific Gravity Bottle.

The Specific Gravity Bottle.—Amongst the most accurate instruments for obtaining the specific gravity of a liquid is the specific gravity bottle (Fig. 1). This consists of a light glass flask, generally made to contain some even quantity of distilled water at 15.5° C. (60° F.), such as 50 or 100 grammes, or 500 or 1000 grains. An accurately ground stopper, perforated by a fine hole, fits into the neck of the little flask. It is so arranged that when the flask, including the perforation in the stopper, is filled with pure water, at the temperature of 15.5° C. (60° F.), the quantity of water in the flask just amounts to one of the weights mentioned above. In using the apparatus the flask is completely filled with the liquid to be examined, and the stopper inserted, care being taken that the liquid has a temperature of 60° F.; the exterior of the flask is then wiped perfectly dry and the whole weighed. The weight of the flask, which must have been previously ascertained (usually a brass counterpoise equal to the weight of the flask is supplied along with it, and this obviates the first part of the next calculation), is now deducted from the total weight; this gives the weight of a volume of the liquid equal to that of the water which the flask holds. The following

calculation then gives the specific gravity of the liquid under examination :

$$\begin{array}{cccc} \text{Weight of} & : & \text{weight of} & : : \text{specific gravity} : \text{specific gravity} \\ \text{water.} & & \text{liquid.} & & \text{of water.} & & \text{of liquid.} \end{array}$$

Supposing the experiment to have been conducted with a flask holding 100 grammes of water, and the weight of the corresponding volume of the liquid under examination is found to be 100.42 grammes, then the calculation, taking the specific gravity of water as 1 (or, as it is commonly expressed, water = 1), is as follows :

$$100 : 100.42 :: 1 : 1.0042.$$

If the specific gravity of water be taken as 100 (water = 100), then the specific gravity of the liquid expressed under these conditions would be 100.42.

In the brewery the specific gravity of water is always taken as 1000 (water = 1000), and the specific gravity of the above liquid, expressed in this way, would be 1004.2.

It is obvious that a flask containing any quantity could be made to serve this purpose, only in such a case a calculation would have to be made each time, whereas, with a flask holding 1000 definite units of weight, the result can be obtained by mere inspection ; or, with a flask which contains 50 or 500 units, the result needs simply multiplying by 20 or 2.

The Hydrometer.—This instrument is the one most frequently used for ascertaining the specific gravities of liquids in manufacturing operations, since it is by far the easiest and most convenient to manipulate. At the same time its indications are not absolutely accurate, but they are sufficiently so for most practical purposes. Its usual form is shown in the accompanying cut, Fig. 2. The instrument is generally made of glass, but sometimes of metal, and consists of an elongated cylindrical bulb, having attached to its upper end a glass tube of larger or smaller diameter according to the delicacy required in the readings of the instrument, and enclosing a scale of paper or ivory. The lower portion is a small glass bulb, partially filled with small shot or mercury. This not only compels the hydrometer to float in an upright position, but also enables the maker to regulate its weight. The action of the instrument depends on the principle “that a body, when floating in a liquid, displaces a volume of the liquid equal to its own weight.” This may be illustrated by taking a piece of perfectly cylindrical glass tube, Fig. 3, and closing its lower end. A mark is made on the tube at some little distance from its top, and the length between this mark and the bottom of the tube divided into twelve equal parts by lines etched on the tube. To the lines figures as shown in the cut are appended. The tube is now loaded with

FIG. 2.—Hydrometer.

shot or mercury until, when placed in water, it sinks to the point marked 10. The volume of water displaced will be equal to that of the tube from figure 10 to its lower extremity, and this volume of water will have the same weight as the tube and its contents have when weighed in air. For example,

FIG. 3.—Experimental Hydrometer.

let the volume of water displaced by the tube when immersed to line 10 be 100 c.c. (cubic centimetres), and as 1 cubic centimetre of water weighs one gramme, the weight of the apparatus will be 100 grammes. If the tube be now placed in a liquid which has a higher specific gravity than water, it will not sink so far as the line 10. When placed in such a liquid, let the tube sink to the line marked 5; then it is obvious that the volume of liquid displaced in this case is only 50 c.c., or half that of the water displaced in the previous experiment. But since this quantity of the liquid must have the same weight as the volume of water previously displaced, and as it is half its volume, 50 c.c. of it must weigh 100 grammes, and a given volume of such a liquid will weigh twice as much as an equal volume of water, or in other words, it has a specific gravity twice that of water. If, on the other hand, the tube be placed in a liquid specifically

lighter than water, it will sink farther than the mark 10. Let it be placed in a liquid in which it sinks to the mark 12. Obviously, then, twelve volumes of such a liquid weigh just as much as ten volumes of water, consequently the specific gravity of the liquid would be $\frac{10}{12}$ that of water. Every line on the scale corresponds to a certain specific gravity, and this can be found in each case by a sum in simple proportion, thus:

Vol. of liquid displaced : vol. of water displaced :: sp. gravity of water : x .

Supposing the tube to sink to the line marked 8, and that we take the specific gravity of water at 1000, then:

$$8 : 10 :: 1000 : 1250.$$

In using this apparatus, we make the converse observation to that which we make with the specific gravity bottle: with the hydrometer we ascertain the *relative volumes* of the *same weight* of liquids; with the specific gravity bottle we ascertain the *relative weights* of the *same volume* of liquids. Obviously, then, by means of the instrument constructed in the before-mentioned way we could roughly determine the specific gravity of many liquids. In order to be able to make the determinations of the instrument more exact, the form shown in Fig. 4 is given to it. The expansion of the stem does not in the least affect the principle of the action of the instrument, so long as we know the relation between the volume of the graduated portion to that of the whole apparatus. Supposing the instrument Fig. 4 weighs 100 grammes, and when immersed in water up to the mark 90 displaces 90 grammes of water, and that, when immersed in water and left to itself, it sinks to the

point 100 and then displaces 100 grammes of water, and that the distance from 90 to 100 is divided into ten equal parts, the specific gravity which corresponds to any of these divisions can be ascertained as in the former case. For instance, let the instrument sink in a liquid to the mark 95, then—

$$95 : 100 :: 1000 : 1052.863.$$

Volumeters.—Instruments made on the principle just mentioned are also termed “volumeters” because they express the volumes of liquids equal to a given weight, *i.e.*, the weight of the instrument. Of such a nature is the volumeter of Gay-Lussac. It is, however, exceedingly inconvenient to have either to make a calculation or to consult a table each time the instrument is used; consequently, instruments are generally graduated so that the reading of the scale gives directly the specific gravity of the liquid under examination; and this form is the one exclusively used by brewers in this country.

Instruments which directly Indicate Degrees of Gravity.—The principle underlying the graduation of all instruments which show degrees of specific gravity directly may be explained in the following manner. Suppose the perfectly cylindrical tube (Fig. 3) to be so weighted that, when floating freely in water, it sinks to the point marked 10; the volume of water displaced by the tube is equal to the volume of the tube from its lower end to 10; call this quantity one volume. Let this distance in the tube be divided into 100 equal spaces and numbered consecutively. From what has been said before, we know that if the tube when immersed in another liquid only sinks half the distance it did in the previous case, or to the point marked 50, then that fluid has twice the specific gravity of water. If, when placed in a third liquid, it only sinks a third, that is, to the point marked 33.3, then such a liquid has three times the specific gravity of water. Hence it follows that the specific

FIG. 4.
Hydrometer.

gravities 1, 2, 3, are proportional to the volumes $\frac{1}{1}, \frac{1}{2}, \frac{1}{3}$; and this may be extended to any degree of fineness by employing closer numbers. Thus specific gravities 1, 1.01, 1.02, 1.03, are proportional to volumes $\frac{1}{1}, \frac{1}{1.01}, \frac{1}{1.02}, \frac{1}{1.03}$. These latter fractions equal respectively the volumes 1, 0.99009, 0.98039, 0.97087; they are, in other words, the reciprocals¹ of the specific gravities; and since the specific gravities are in arithmetical progression, it follows that the figures for the corresponding volumes are in harmonical² pro-

¹ The reciprocal of a number is the quotient obtained by dividing unity by that number.

² Numbers are said to be in arithmetical progression when they increase or decrease by equal differences, as 0, 3, 6, 6, 12. When a series of numbers are in harmonical progression, the ratio between the first and third of any three contiguous numbers is

gression. When, therefore, a hydrometer is so graduated as to show directly specific gravities, its graduations are the reciprocals of the specific gravities it is constructed to indicate, expressed as fractions of the volume of water which it displaces when allowed to float freely in that liquid. As these numbers are in harmonical progression, the intervals of such a scale will not be equal, but become gradually closer together at that portion of the scale which indicates the higher gravities. This may be readily seen on observing the scale of a hydrometer, Fig. 2 (page 17). This inequality of the intervals between the divisions of the scale becomes less and less apparent as the indications of the instrument increase in delicacy; but it is obvious that they must exist even in instruments constructed to show very minute differences in specific gravity.

Hydrometers which Indicate Pounds per Barrel.—These instruments in reality only indicate specific gravity in another way. Long's saccharometer, which may be taken as an illustration, is made of metal, and has a flat stem, on the opposite sides of which are two scales, the one reading from 0 (specific gravity of water) to 25 lb. per barrel, the other commencing with 25 lb. per barrel and extending up to 52 lb. When the density of the liquid being examined exceeds 25 lb. per barrel, a small weight is attached to the lower part of the instrument, and the readings are then taken from the second scale. As we shall recognise later on, the expression "pounds per barrel" is an exceedingly convenient one for use in the brewery.

Relation of Pounds per Barrel to Specific Gravity.—A barrel is equal to 36 gallons, and as a gallon of water weighs 10 lb., a barrel of water will weigh 360 lb. A barrel of another liquid, such as wort, which has a higher specific gravity than water, will weigh more than 360 lb. On taking the density of such a liquid with Long's saccharometer, or a similar instrument indicating pounds per barrel, we ascertain how many more pounds a barrel or 36 gallons of such liquid weighs over and above 360 lb. Supposing a sample of wort, when tested in this manner, shows 18 lb. per barrel, a barrel of such a wort will weigh $360 + 18 = 378$ lb. Its specific gravity may be found by the following sum in proportion:

360	:	378	::	1000	:	1050
Weight of barrel		Weight of barrel		Weight of 1000 parts		Weight of 1000 parts
of water.		of wort.		of water.		of wort.

Conversely, the weight of a barrel of wort may be found from the specific gravity in the following manner:

$$1000 : 1050 :: 360 : 378$$

and $378 - 360 = 18$ lb.

Or the weight per barrel may also be found from the specific gravity in the following manner, where the weight in excess of the 1000 is employed:

$$1000 : 360 :: 50 : 18.$$

As $360 \div 1000 = 0.36$, the same result is obtained by multiplying the 50 by as the difference between the first and second is to the difference between the second and third; thus the figures, 2, 3, 6 are in harmonical progression, and consequently

$$2 : 6 :: 3 - 2 : 6 - 3.$$

0.36 thus, $1050 - 1000 \times 0.36 = 18$ lb. per barrel. The converse operation, conversion of pounds per barrel to specific gravity, may be effected by dividing the number of pounds per barrel by 0.36, and adding the sum thus obtained to 1000, as $18 \div 0.36 = 50$, and $50 + 1000 = 1050$. The number 36, being the number of gallons contained in the barrel, is one easily remembered. Specific gravity, when expressed in the usual manner (*i.e.*, water = 1000), may be regarded as the weight in excess in pounds over and above the weight of a hundred gallons of water, that is, 1000 lb., just as pounds per barrel are the excess in weight over and above 36 gallons (or 360 lb.) of water.

Hydrometers which Indicate directly the Percentage of Substance in Solution.—Of this nature is the hydrometer of Balling, almost exclusively used in Continental breweries. Its indications show how many parts of cane-sugar are contained in 100 parts by weight of a solution at a temperature of 14° R. (63.5° F.). It may seem surprising that a hydrometer indicating percentages of cane-sugar should be used in brewing operations, instead of one which indicates percentages of brewers' extract. It has, however, proved so difficult to ascertain what the true percentages of brewers' extract corresponding to the various degrees of gravity are, that it is found advisable to take some readily accessible substance, a solution of which can be easily prepared of any required strength. Though the instrument does not express true amounts of brewers' extract, yet its indications are strictly comparable amongst themselves, and this is the question of real importance to the brewer. On the Continent, therefore, the gravity of a wort is usually expressed in percentages of extract according to Balling's scale, and the amount of gravity lost during fermentation in a similar manner.

Tralles' Alcoliolimeter.—This instrument is also constructed on an empirical scale, and indicates directly the amount of alcohol by volume contained in mixtures of alcohol and water at a temperature of 60° F.

Bates's Saccharometer.—It is obvious that the length of the scale of any hydrometer of a convenient size is limited, if its divisions are to be so wide apart that they can be read with accuracy. Consequently, in order to deal with the various specific gravities of the fluids met with in the brewery, it is necessary to have several instruments with different scales. This, though of no importance when the hydrometers are kept in one room, becomes a positive disadvantage when they have to be carried about from place to place. A number of attempts have been made to obviate this inconvenience and to contrive a single instrument by means of which, with slight alterations, all the gravities met with in the brewery may be measured. The problem was satisfactorily solved by Bates, who constructed an instrument provided with five removable weights, or "poises," as they are termed, and capable of estimating the specific gravity of any liquid from 1000° to 1150° (water = 1000). This is the instrument universally employed by the Excise Authorities, and also very frequently by brewers.

Bates's saccharometer is constructed of brass, and has much the same form

as an ordinary hydrometer, but the stem is square instead of cylindrical. In place of the lower bulb, which in the ordinary hydrometer is weighted with mercury or shot, there is a ring to which can be attached in turn, by means of a conical hole in its under side, a number of poises of different weights and bulks, each of which is provided with a conical peg. In this way a portable instrument, reading from 1000° to 1150° of specific gravity (water = 1000), is obtained. The scale has thirty principal divisions divided into halves, and, with the smallest poise attached, the instrument indicates degrees of gravity from 1000 to 1030; that is, the reading of the scale *plus* 1000. The other poises are marked respectively 30, 60, 90, 120, and, when any one of these is attached to the instrument, the number marked on the poise is added to the reading of the scale and their sum added to the 1000. Thus, if the instrument with the poise marked 60 attached, is immersed in a liquid and sinks to the mark 15 on its scale, then the specific gravity indicated will be $15 + 60 + 1000 = 1075^{\circ}$. The principles which underlie the construction of the instrument may be explained in the following manner. Suppose that an instrument has its stem so divided into thirty divisions that these indicate degrees of specific gravity from 1000 to 1030, and that the instrument weighs 103 grammes. It is evident that when immersed in water it will displace 103 c.c. (1 cubic centimetre of water weighs one gramme), and that when placed in a liquid of specific gravity 1030 it will displace 100 c.c. If to the instrument a poise be attached which has a bulk of 3 c.c. and a weight of 6.18 grammes, it will sink to its zero mark when immersed in a liquid of specific gravity 1030, because the total bulk of the instrument is now 106 c.c. and this multiplied by $1.03 = 109.18$ grammes. When immersed in a liquid of specific gravity 1060 it will sink to the point marked 30, since it then displaces 103 c.c. of liquid and $103 \times 1.06 = 109.18$ grammes. This may also be shown to be true of every other division on its scale. If another poise having a volume of 6 c.c. and weighing 12.54 grammes is attached, then the instrument registers from 1060 to 1090 of specific gravity, for either 109 c.c. of a liquid of specific gravity 1060, or 106 c.c. of a liquid of specific gravity 1090, weigh 115.54 grammes. It is obvious that this process can be extended each successive poise having an additional volume of 3 c.c. and a constantly increasing weight. The following considerations will show how this weight is obtained:—Suppose that without increasing the original volume of the instrument (103 c.c.), we wish to make it sink to zero in a liquid of a specific gravity of 1030, we shall have to increase its weight by $(103 \times 1.03) - 103 = 3.09$ grammes. If we add on a volume of 3 c.c. this will displace $3 \times 1.03 = 3.09$ grammes, consequently this weight will have to be added to the instrument in addition to the former one to cause it to sink to zero, and $3.09 + 3.09 = 6.18$ grammes. The weights of the other poises may be found in a similar manner. It is obvious that whatever the original volume of the instrument may be, the respective volumes and weights of the poises may be obtained from the above data by simple proportion.

CHAPTER II

CHEMISTRY, WITH SPECIAL REFERENCE TO THE MATERIALS USED IN BREWING

BEFORE entering into a detailed description of the chemistry of the materials used in brewing, it will be well to devote a short space to the consideration of a few of the fundamental principles of chemistry.

The Elements.—The chemist is able to resolve most of the forms of matter which occur in nature into two or more substances. This process is called “chemical analysis.” After continuing this for a time, a stage is reached in which a variety of bodies are obtained which resist all attempts at further subdivision. Suppose the chemist takes a piece of chalk ; he is readily able to resolve this into calcium, carbon, and oxygen ; but he can go no further than this, for, however much chemists have endeavoured to split up calcium, carbon, or oxygen into other bodies, their efforts have so far been unsuccessful. Substances of this nature, which it has been found impossible to further chemically subdivide, are called “elements.”

Working in this way, chemists have been able to discover some 78 different elements. Comparatively few of these, however, concern the brewer.

Chemical Symbols.—In describing the elements, it is customary to indicate each of them by a symbol. This is either the first letter or the first two letters of the Latin name of the element. Thus H is used to denote hydrogen, O oxygen, Ca calcium, Cl chlorine, &c. In most instances the initial letters of both the English and Latin names of the elements are identical : in a few, as, for instance, K (kalium) potassium, Na (natrium) sodium, &c., they are not.

Combining Proportions and Atoms.—The view that matter is made up of exceedingly minute particles was held even in the earliest times by the Hindoo, Phœnician and Egyptian philosophers. In modern times the idea was maintained by Newton and opposed by Descartes, Leibnitz and Euler, who believed in the infinite divisibility of matter. It cannot, however, be said to have attained a workable form until John Dalton, early in the nineteenth century, formulated his celebrated atomic theory to account for the phenomena of chemical combination in definite and multiple proportion. When elements combine with each other, the combination always takes place in some definite relative weight or multiple of that weight. Thus when

oxygen and calcium combine, it is always in the proportion of 16 parts by weight of oxygen and 40 parts of calcium. The number 16 is called the atomic weight of oxygen, and 40 that of calcium. The symbol is, however, used to express something beyond the mere name of the element; it not only denotes the element, but its atomic weight as well. Thus, when we write FeO (ferrous oxide), we not only express a compound of iron and oxygen, but also at the same time signify that the compound consists of one atom or 56 parts by weight of iron, and one atom or 16 parts by weight of oxygen. If we have 72 ounces of ferrous oxide, we know at once from its formula that it consists of 56 ounces of iron in combination with 16 ounces of oxygen; or if it were 72 pounds, tons, or grains, the other figures would be respectively pounds, tons, or grains. In such a compound as water, where two atoms of hydrogen are united to one atom of oxygen, a small figure 2 is appended to the symbol for hydrogen, H_2 ; H_2O therefore denotes that water is composed of two atoms of hydrogen combined with one atom of oxygen. Similarly NH_3 (ammonia) stands for one atom of nitrogen combined with three atoms of hydrogen; CH_4 (methane), one atom carbon and four atoms hydrogen.

Table of the more Common Elements.—The following table contains a list of those elements which are principally met with in brewing chemistry, together with their symbols and approximate atomic weights:

Hydrogen H	1	Sulphur S	32
Carbon C	12	Chlorine Cl	35.5
Nitrogen N	14	Potassium (Kalium) . . K	39
Oxygen O	16	Calcium Ca	40
Sodium (Natrium) . . Na	23	Iron (Ferrum) . . Fe	56
Magnesium Mg	24	Copper (Cuprum) . . Cu	63.6
Aluminium Al	27	Bromine Br	80
Silicon Si	28	Silver (Argentum) . . Ag	108
Phosphorus P	31	Iodine I	127

Valency.—Another characteristic of the atoms of the elements is that with some of them the atom is capable of uniting with one atom of another element, as hydrogen, which only forms one compound with chlorine, hydrochloric acid gas, HCl . Such elements are termed “univalent.” The atom of others is able to unite with several atoms of another element, as we have just seen in the case of water, where one atom of oxygen unites with two atoms of hydrogen. Hence the element oxygen is termed “bivalent.” “Trivalent” nitrogen is able to unite with three atoms of hydrogen to form NH_3 (ammonia), and “quadrivalent” carbon can unite with four hydrogen atoms to form methane, CH_4 . This atom-fixing power is called “valency” or “equivalence,” and the greatest number of hydrogen atoms, or of any other univalent element, which any particular element can fix is taken as its standard of valency. In the case of such an element as calcium, which does not combine directly with hydrogen, we are compelled to ascertain its valency indirectly. Calcium combines readily with oxygen to form calcium oxide, CaO , and since,

so far as valency is concerned, one atom of oxygen is equivalent to two atoms of hydrogen, calcium must be bivalent.

Bonds.—It is exceedingly convenient to be able to give visible expression to the valencies or atom-fixing powers of elements, and for this purpose short lines drawn from the symbol of the element, technically called “bonds” or “bonds of affinity,” are frequently made use of. Thus one short line affixed to the symbol H— tells us that the hydrogen atom possesses one bond of affinity; three similar lines to nitrogen —N— that it has three, $\begin{array}{c} | \\ -\text{C}- \\ | \end{array}$ that carbon has four, and so on with the rest of the elements.

Chemical Union.—When two atoms enter into chemical combination with each other, the bonds of the one are supposed to become linked with those of the other. Thus in the compound H—Cl, the bond of the hydrogen atom is linked to the bond of the chlorine atom; in water H—O—H the two bonds of the oxygen atom are linked to the bonds of the two hydrogen atoms. Similarly,

In order to picture more vividly the valency of elements, and the way in which their bonds of affinity unite, small differently coloured balls, with short pegs affixed to them, are often employed. Thus hydrogen may be represented by a white ball with one peg, chlorine by a yellow ball with one peg, oxygen by a red ball with two pegs, nitrogen by a blue ball with three pegs, and carbon by a black ball with four pegs. The pegs are easily connected by small pieces of rubber tubing. The following compounds illustrated in this way appear thus:

FIG. 5.—Valency of Elements.

Constitution.—Any plan of this kind which gives us an idea as to the position in which the various atoms of a compound are arranged with reference to one another is called a “structural” or “constitutional formula”; that is, it expresses the structure or constitution of the compound.

It must be distinctly borne in mind that all this doctrine of valency and linkage of atoms is purely hypothetical. It has, however, proved itself of

such immense assistance in explaining the way in which compounds are built up or pulled to pieces, that it may fairly be considered that some such arrangement of the atoms really exists, though we are by no means certain as to what its exact nature may be.

Saturated Compounds.—When, as in the cases above mentioned, *all* the bonds of affinity of an element are linked with those of one or more other atoms, such an atom is said to be “satisfied.” Thus a univalent atom requires one univalent atom to satisfy it, as in H—Cl (hydrochloric acid gas). A bivalent atom requires either two univalent atoms or one bivalent atom, as in calcium chloride Ca=Cl_2 , or calcium oxide Ca=O . Similarly, a trivalent atom requires three univalent, and a quadrivalent atom four univalent or two bivalent atoms to satisfy it, as in—

Such compounds as the above, in which all the bonds of affinity of the atoms are satisfied, are known as “saturated compounds.” They are possessed of considerable stability, and are incapable of taking up more atoms.

Unsaturated Compounds.—In such compounds as carbon monoxide, $=\text{C=O}$, two of the bonds of the carbon atom are unsatisfied, and as this latter pair of bonds are ready to attach themselves to other atoms in order to become satisfied, the resulting compound is unstable and prone to change.

Thus the simple ignition of carbon monoxide gas in air affords the unsatisfied bonds of the carbon the opportunity of attaching themselves to an atom of oxygen to form the saturated compound carbon dioxide, O=C=O . Such compounds as carbon monoxide are said to be “unsaturated.”

Molecules.—When two or more atoms combine to form a definite substance, the smallest chemical mass of that substance is called a “molecule.” Thus, HCl , H_2O , CH_4 , represent molecules. The molecule is considered to be the smallest mass of matter which can exist in a free state.

From this it is inferred that the elements do not exist in the state of atoms, but as molecules, two or more atoms being always combined, so that the bond or bonds of affinity of the one atom satisfy the bond or bonds of the other. Thus, hydrogen is supposed to exist as H—H , oxygen as O=O , and so on with the rest of the elements.

But many chemical reactions are known in which an interchange of atoms takes place. For instance, when the metal sodium is placed in contact with water, the following reaction ensues:

In this, one of the hydrogen and one of the oxygen atoms of each molecule of water unite with one of the sodium atoms to form a molecule of sodium

hydroxide. The remaining hydrogen atoms of the molecule of water are liberated, and unite to form a molecule of hydrogen. The atom is considered to be the smallest particle of an element which is capable of taking part in a chemical reaction.

Nascent State.—When, as in the case just mentioned, an element is liberated from a compound in atoms, it is said to be in the nascent state, and in this condition it manifests a degree of activity which it does not exhibit under ordinary circumstances. For instance, hydrogen gas may be passed through a dilute solution of nitric acid (HNO_3) without in the slightest degree affecting the composition of the acid. But if hydrogen be generated in such a solution, the chemical composition of the acid becomes entirely changed. The hydrogen, under these circumstances, is able to combine with and remove the whole of the oxygen of the nitric acid as water, whilst at the same time two other atoms of hydrogen attach themselves to the nitrogen to form ammonia. In this way nitric acid is converted into ammonia according to the following equation :

Other elements, such as oxygen, nitrogen, &c., behave in a similar manner.

Chemistry of the Carbon Compounds.—By far the greater number of substances with which we have to deal in the operation of brewing have at one time entered into the structure of and been formed by living organisms. Hence they belong to that domain of chemistry which was at one time called organic. Formerly the opinion was held that the so-called organic compounds were of a nature essentially different from the inorganic. Though the chemist could readily build up the latter class of bodies from the elements, he had been, up to that time, unable to form any of the substances belonging to the former class in a similar manner.

This view received its death-blow in 1828, when Wöhler produced urea artificially. Since then it has been found possible to build up in the laboratory an enormous number of substances which it was formerly supposed could only be formed by living organisms. Even such complex bodies as the sugars have been built up from the elements in recent times, and it is now fully recognised that, chemically, there is no difference between organic and inorganic compounds. They are composed of the same elements, and these obey the same laws of chemical combination in either class of bodies.

Since the element carbon enters into the composition of all the so-called organic bodies, that which was formerly termed “organic chemistry” is generally spoken of now as “the chemistry of the carbon compounds.”

Empirical Formula.—In investigating a new carbon compound the first thing to be done is to establish its elementary composition. When the results are expressed centesimally and each of the values is divided by the respective

* A large numeral placed before a symbol or group of symbols means that the symbol or group is to be multiplied by that number. Thus, in this equation 8H stands for eight atoms of hydrogen, and $6\text{H}_2\text{O}$ for six molecules of water.

atomic weights of the elements, the atomic ratios of the elements in the compound are obtained. To make this quite clear we may cite an analysis of acetic acid. This gives the following values expressed centesimally.

Carbon	40.0	per cent.
Hydrogen	6.6	"
Oxygen (by difference)	53.4	"
	<u>100.0</u>	"

Dividing these values by the atomic weights of the respective elements, the quotients are as follows :

$$\frac{40.0}{12} = 3.3 \qquad \frac{6.6}{1} = 6.6 \qquad \frac{53.4}{16} = 3.3.$$

Therefore the relative number of atoms of carbon, hydrogen and oxygen in acetic acid is: 3.3 : 6.6 : 3.3 or 1 : 2 : 1. Consequently the simplest formula expressing the composition of acetic acid is CH_2O .

Molecular Formula.—The empirical formula is not of necessity identical with the molecular formula. To establish this other means must be resorted to. There are several methods of determining the molecular weight of a compound which will now be briefly described.

1. *The Chemical Method.*—This is applicable to all substances, although it does not always lead to definite conclusions. It consists in preparing derivatives and comparing their formulæ with that of the substance under investigation. It is an easy method in the case of an acid or base, for then it is only necessary to prepare a salt and determine the amount of metal or acid in combination with the acid or base. Let us take the case above cited, acetic acid. Silver acetate is found to contain 64.6 per cent. of silver. As the atomic weight of silver is 108 the amount of the other constituent combined with one atom of silver is found from the proportion

$$\frac{(100 - 64.6) \times 108}{64.6} = 59$$

Since in silver acetate one atom of the hydrogen of acetic acid is replaced by one atom of silver, it follows that the molecular weight of acetic acid must be $59 + 1 = 60$. That is, however, if acetic acid is a monobasic acid, which we know is the case from the fact that it does not form a salt containing more silver than the one just mentioned.

2. *The Vapour Density Method.*—This method of determining molecular weights is limited to compounds which can be volatilised without undergoing decomposition. It is founded on the law of Avogadro, according to which equal volumes of all gases at the same temperature and pressure contain an equal number of molecules. The molecular weights of all gases, therefore, bear a constant relation to their specific gravities. The molecular weight of hydrogen being 2, that of any compound is obtained by multiplying its specific gravity in the state of gas compared with hydrogen by 2.

3. *Determination of the Molecular Weights of Substances when in Solution.*—This is done by determining (a) the osmotic pressure ; (b) the lowering of

the vapour pressure or raising of the boiling-point; (c) the lowering of the freezing-point. Further remarks on molecular weight will be found on p. 58.

Isomeric Compounds.—Many instances are met with among carbon compounds of substances having the same composition but different properties. These were called by Berzelius isomerides (*ἴσος* equal, and *μέρος* part). Compounds which are not only identical in composition, but also in molecular weight, are said to be “metameric.”

The elements which enter into the composition of organic substances are, as a rule, extremely limited in number, being generally confined to not more than five; yet the manner in which, by the operation of a few simple laws, these few elements are varied in their relations one with another, and the enormous number of different substances which are produced in this way, naturally or artificially, is truly remarkable. One of the most striking features of this department of chemistry is the numerous series of compounds which are constructed on a definite plan common to all its members, each of which merely differs from the member preceding it as we proceed down the series by some simple relation, such as containing one atom of carbon and two atoms of hydrogen more. Such series are called “homologous” (*ὁμός*, like, *λογος*, description), because all the members of any particular series being constructed on the same plan, the same description applies to each.

The first of these series, the paraffins, is a particularly important one, for it forms the foundation of many other homologous series which contain bodies of special interest to the brewer. We shall find as we proceed that the paraffins are, as it were, the skeletons of such bodies as the alcohols, the sugars, and many other substances.

The Paraffins.—The first member of this series is methane, $\begin{array}{c} \text{H} \\ | \\ \text{H}-\text{C}-\text{H} \\ | \\ \text{H} \end{array}$

a body in which the four bonds of the carbon atom are each linked with an atom of hydrogen, and as all the bonds of the carbon atom are satisfied in the above compound, a molecule of considerable stability, and one which is incapable of attaching further atoms to itself, results.

The bonds of the carbon atoms have the power of satisfying one another under certain conditions, and by virtue of this property a series of bodies of the following constitution is formed:

and so forth, in which the carbon atoms are linked together in what is termed an “open chain.”

In this way the second and the remaining members of the paraffin series are progressively built up:

Methane	CH ₄	Butane	C ₄ H ₁₀
Ethane	C ₂ H ₆	Pentane	C ₅ H ₁₂
Propane	C ₃ H ₈	Hexane	C ₆ H ₁₄

Each member of the series as we descend contains one carbon and two hydrogen atoms (CH₂) more than the preceding one. The list does not stop at hexane; all the members of the series from methane CH₄ to hexadecane C₁₆H₃₄ are known. The whole of the members of the series may be represented by the general formula, C_{*n*} H_{2*n* + 2}, in which *n* represents the number of carbon atoms in the molecule.

Substitution.—The paraffins being fully saturated, show no inclination to combine directly with other bodies, but they exhibit in a marked degree the curious property—shared by most organic compounds—of allowing one or more of their hydrogen atoms to be replaced by a univalent element or group. This process is called “substitution.” For instance, it is possible to replace successively the hydrogen atoms in methane by the univalent element chlorine, and obtain in this manner the following four bodies:—

Unsaturated Groups.—Many unsaturated groups are met with, such as H—O—(hydroxyl), which body may be regarded as water H—O—H *minus* one atom of hydrogen. Such groups are incapable of independent existence, but often exist in pairs, the free bond or bonds of the one group satisfying those of the other; thus hydrogen peroxide H—O—O—H is a well-known compound.

But the property of these groups which chiefly concerns us is their power of replacing the elements, and thus forming what are termed “substitution compounds.” The univalent group H—O—(hydroxyl) often takes the place of an atom of hydrogen, as, for instance, when it replaces one of the hydrogen

atoms in methane, and yields $\begin{array}{c} \text{H} \\ | \\ \text{H}-\text{C}-\text{O}-\text{H} \\ | \\ \text{H} \end{array}$ or methylic alcohol.

Compound Radicles.—Unsaturated groups, which, so far as substitution is concerned, behave in this way, are often called “compound radicles.” The members of the paraffin series, for instance, when deprived of one atom of hydrogen, form such radicles. Of such a nature are methyl CH₃, that is methane, CH₄ *minus* one atom of hydrogen; ethyl C₂H₅ which is similarly related to ethane C₂H₆, and so on. Obviously such bodies cannot exist in the free state.

The Alcohols.—When one of the hydrogen atoms of a paraffin is replaced by a hydroxyl group we obtain a monohydric alcohol. In this way the homologous series of alcohols, of which methylic alcohol forms the first member, is produced, each member differing from its predecessor by CH_2 . The following are the first six members :

Methylic alcohol (or carbinol)	CH_3OH	Butylic alcohol	$\text{C}_4\text{H}_9\text{OH}$
Ethylic alcohol (or methylcarbinol)	$\text{C}_2\text{H}_5\text{OH}$	Amylic alcohol	$\text{C}_5\text{H}_{11}\text{OH}$
Propylic alcohol	$\text{C}_3\text{H}_7\text{OH}$	Hexylic alcohol	$\text{C}_6\text{H}_{13}\text{OH}$

The Dihydric Alcohols and Glycols.—When two atoms of the hydrogen of a paraffin are replaced by three hydroxyl groups, we obtain another homologous series of alcohol, known as the “glycols.”

Thus ethane—

$\text{CH}_3(\text{OH})$, and propane, $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_3$, gives propylene glycol, $\text{CH}_2(\text{OH}) \cdot \text{CH}_2 \cdot \text{CH}_2(\text{OH})$.

The Trihydric Alcohols.—When three of the hydrogen atoms of a paraffin are replaced by three hydroxyl groups, another homologous series of alcohols is produced of which glycerol (glycerin) is the first member; thus

$\text{CH}_2\text{OH} \cdot \text{CHOH} \cdot \text{CH}_2\text{OH}$ glycerol. Other members of the series are known.

Tetrahydric and Pentahydric Alcohols are also known.

Hexahydric Alcohols.—By replacing six of the hydrogen atoms in the paraffin hexane C_6H_{14} , by hydroxyl groups, the following alcohol, mannitol, is obtained—

The compound is found in the drug manna, which is excreted by certain tropical trees. Sorbitol, though a different body, has the same composition; it is what is termed an optical stereoisomeride (*see* page 40).

The Aldehydes.—Most of the monohydric alcohols, when they are submitted to the action of certain oxidising agents (such as potassium dichromate and sulphuric acid), have two atoms of hydrogen removed from their molecule, and a body called an “aldehyde” (*alcohol dehydrogenatum*) results. Methylic alcohol when passed over a red-hot spiral of platinum wire yields methylic aldehyde or formaldehyde—

Similarly, ordinary alcohol yields ethylic or acetic aldehyde (ordinary aldehyde)—

Thus the aldehydes form another homologous series. They frequently behave like unsaturated bodies, as if one bond of the C and one of the O in the CHO group were unsatisfied. These unsatisfied bonds exhibit an affinity for oxygen, and in virtue of this are able to reduce many metallic oxides. They also form compounds with phenylhydrazine (*see* page 37).

The Aldoses.—These bodies are of an aldehydic nature; they contain the group CHO, reduce alkaline metallic solutions, and yield characteristic crystalline compounds with phenylhydrazine. They are usually sweet to the taste, often optically active, and some of them are capable of undergoing alcoholic fermentation. The names triose, tetrose, &c., have been proposed by E. Fischer for these bodies, according to the number of carbon atoms they respectively contain, whilst the general name of “aldose” for the whole group was suggested by Professor H. E. Armstrong. The following are among the known members :

Triose.	. . .	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CHO}$	. . .	Glycerose, or glyceric aldehyde.
Tetrose	. . .	$\text{CH}_2\text{OH}\cdot 2(\text{CHOH})\cdot\text{CHO}$	. . .	Erythrose.
Pentose	. . .	$\text{CH}_2\text{OH}\cdot 3(\text{CHOH})\cdot\text{CHO}$	. . .	Arabinose, xylose.
Hexose	. . .	$\text{CH}_2\text{OH}\cdot 4(\text{CHOH})\cdot\text{CHO}$	. . .	Glucose, galactose, mannose, &c.
Heptose	. . .	$\text{CH}_2\text{OH}\cdot 5(\text{CHOH})\cdot\text{CHO}$		
Octose	. . .	$\text{CH}_2\text{OH}\cdot 6(\text{CHOH})\cdot\text{CHO}$		
Nonose	. . .	$\text{CH}_2\text{OH}\cdot 7(\text{CHOH})\cdot\text{CHO}$		

Of these, the pentoses and hexoses are of particular interest to the brewer. The pentoses he meets with to a certain extent in his materials, and to the hexoses or their derivatives belong all the sugars which he subjects to the process of alcoholic fermentation. It is a remarkable fact that of the known aldoses only those which contain three carbon atoms, or a multiple of three, are fermentable by brewers' yeast—that is, the trioses, hexoses, and nonoses.¹ The pentose sugar arabinose, though unfermentable by ordinary yeast, is attacked and broken down by an organism, the *Bacillus ethaceticus*, yielding ethyl alcohol, acetic acid, carbon dioxide, hydrogen, and traces of succinic acid.

The Ketones.—This homologous series of bodies may be regarded as derivatives of the aldehydes, in which the hydrogen atom of the CHO group is replaced by a compound radicle derived from a paraffin. Thus when the hydrogen atom in aldehyde is replaced by CH_3 (methyl), acetone is formed—

The group CO (carbonyl) is the distinguishing characteristic both of the

¹ This property is only possessed by nono-mannose, nonoglucose being unfermentable.

aldehydes and of the ketones. Mixed ketones are known, such as the methyl ethyl ketone, $\text{CH}_3\cdot\text{CO}\cdot\text{C}_2\text{H}_5$.

Ketoses.—These bodies bear the same relation to the ketones that the aldoses do to the aldehydes. The simplest known ketose is dihydroxyacetone, $\text{CH}_2\text{OH}\cdot\text{CO}\cdot\text{CH}_2\text{OH}$. Fructose (lævulose), one of the constituents of malt and of invert sugar, is of this nature. It has the following constitution :

and may be considered as derived from butyl-methyl ketone,

Cyanhydrins are produced by the direct union of hydrocyanic acid with an aldehyde or a ketone, thus :

Similar compounds are formed by the aldoses and ketoses, thus :

When the cyanhydrins are acted upon by a mineral acid, the CN group resolves itself into the COOH group, ammonia being formed simultaneously, thus :

This is an exceedingly interesting reaction, since it enables us to pass from a lower to a higher compound. In the instance just given we pass from arabinose to mannose.

Acetic Acid Series.—When the two free bonds of an aldehyde derived from a paraffin are satisfied with an atom of oxygen, we obtain a series of acids of which formic acid is the first member, thus :

Ordinary aldehyde similarly yields acetic acid :

The aldoses behave in a similar manner, yielding the corresponding acids of the series. Thus glucose yields gluconic acid :

Conversion of Acids into Aldehydes and Alcohols.—It will be seen that it is possible by a process of gradual oxidation to convert an alcohol

first into an aldehyde, and then by further oxidation to convert the aldehyde into an acid. By operating in the reverse way, that is to say, if, instead of using an oxidising, we use a deoxidising agent, or what is commonly called a "reducing" agent, we can, so far as the acids corresponding to the aldoses are concerned, reverse the operation. Thus, if gluconic acid be acted upon at a low temperature by nascent hydrogen, which may be readily generated from sodium amalgam,¹ the acid is first converted into the aldose in the following manner:

By continuing the process two atoms of hydrogen are added on to the CHO group, and the alcohol, which contains the CH_2OH group, then results.

Lactones.—Here may be mentioned a peculiar tendency possessed by certain acids, amongst which are those derived from the sugars, of losing a molecule of water and becoming converted into a lactone when evaporated to a strong aqueous solution. Thus gluconic acid, $\text{CH}_2\text{OH}\cdot 4(\text{CHOH})\cdot \text{COOH}$, becomes gluconic lactone, $\text{CH}_2\text{OH}\cdot \text{CHOH}\cdot \text{CH}_2\cdot 2(\text{CHOH})\cdot \text{CO}$. These bodies have a

$\begin{array}{c} \text{O} \\ \text{—} \end{array}$

neutral reaction.

Lactic Acid.—The series of acids to which this substance (frequently met with in fermentations) belongs is closely allied to the acetic series. When, in the third member of that series, propionic acid, $\text{CH}_3\cdot \text{CH}_2\cdot \text{COOH}$, one of the hydrogen atoms of the middle carbon atom, is replaced by hydroxyl, lactic acid, $\text{CH}_3\cdot \text{CH}(\text{OH})\cdot \text{COOH}$, is formed.

Oxalic Acid is derived from the first dihydric alcohol or glycol by oxidation of the two CH_2OH groups to COOH , thus:

It is one of the acids occurring in malt, and is often seen in the form of calcium oxalate, which forms very characteristic octahedral crystals, in specimens of yeast when examined under the microscope.

Calcium oxalate, together with a small amount of organic matter, frequently deposits on the sides and on the tempering coils of the fermentation vessels.

Succinic Acid.—This acid, which is an invariable product of alcoholic fermentation, is derived from the third member of the glycol series, butylene glycol, $\text{CH}_2\text{OH}\cdot \text{CH}_2\cdot \text{CH}_2\cdot \text{CH}_2\text{OH}$, which, when oxidised, yields succinic acid, $\text{COOH}\cdot \text{CH}_2\cdot \text{CH}_2\cdot \text{COOH}$.

Salts of Organic Acids.—All the organic acids share with the mineral acids the property of combining with bases to form salts. Thus when

¹ Made by warming 100 grammes of mercury in a porcelain dish, covering the surface with a layer of solid paraffin melted, and plunging into the mercury (in pieces about the size of a pea, one at a time) about 5 grammes of metallic sodium.

sodium hydroxide and acetic acid are brought together, the following reaction ensues :

In other words, the hydrogen of the COOH (carboxyl) group can be replaced by a metal.

Bibasic Acids.—Acids, such as oxalic and succinic, which possess two COOH groups, are able to combine with two atoms of a univalent or with one atom of a bivalent base. Thus we have COONa·COONa, sodium oxalate ;

and $\begin{array}{c} \text{COO} \\ \diagup \\ \text{Ca} \\ \diagdown \\ \text{COO} \end{array}$, calcium oxalate. They also form acid salts in which the hydrogen of one of the COOH groups only is replaced by a base, as in acid oxalate of potassium, COOH·COOK.

Compound Ethers¹ or Esters.—These bodies are analogous in constitution to the salts just mentioned. In their formation the alcohols behave in the same manner as the metallic hydroxides. Ethylic alcohol may be regarded as the hydroxide of ethyl, C₂H₅(OH), just as caustic soda is the hydroxide of sodium, Na(OH), and both can be made to combine with an acid in a similar manner, thus :

The reaction does not take place so readily as with a metallic base, consequently the process which has to be adopted to cause such a combination is much more complicated.

Many of the esters are extremely fragrant bodies, and on their presence depends the flavour of many fruits, the bouquet of wines, and to some extent the flavour of beer. Vinegar chiefly derives its fragrant odour from the presence of a small amount of ethylic acetate. The pear flavour of the confectioner is amylic acetate ; pine-apple flavour is ethylic butyrate.

When an ester is boiled with an alcoholic solution of a metallic hydroxide, such as caustic soda, the following decomposition ensues :

This reaction, which is typical of hydrolysis or saponification, is used for the quantitative determination of the esters, since it is comparatively easy to

¹ The esters or compound ethers must not be confounded with the ordinary ethers. These bear the same relation to the metallic oxides as the alcohols do to the hydroxides.

Thus ordinary ethylic ether has the constitution $\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{O} \\ \diagdown \\ \text{C}_2\text{H}_5 \end{array}$ just as sodium oxide is $\begin{array}{c} \text{Na} \\ \diagup \\ \text{O} \\ \diagdown \\ \text{Na} \end{array}$.

In order to avoid confusion between the two bodies the compound ethers are now generally called esters.

ascertain the amount of alkali neutralised by the acid liberated in the decomposition.

The Amides.—Another univalent group, NH_2 (amidogen), often replaces a hydrogen atom, or the hydroxyl group, in organic compounds, and the body so formed is called an “amide.” When in an organic acid the hydroxyl group of the $\text{CO}(\text{OH})$ group is replaced by NH_2 , an acid amide is formed. Acetamide, $\text{CH}_3\cdot\text{CO}(\text{NH}_2)$, is such a body.

If one of the hydrogen atoms of an organic acid, in combination with carbon be replaced by NH_2 an amino acid is produced. As an example of one of

these compounds, amino-acetic acid, $\text{H}-\overset{\text{H}}{\underset{\text{NH}_2}{\text{C}}}-\text{COOH}$, in which one of the H

atoms of the CH_3 group of acetic acid is replaced by amidogen¹ may be cited.

Asparagine.—This is the acid amide of amino-succinic acid. The relations of the three bodies are shown in the following formulæ:²—

Asparagine was first discovered in asparagus, hence its name; it is found in most germinating plants. It forms large white transparent crystals, and is a highly diffusible substance. Young seedlings of the *Lupinus luteus*, when grown in the dark to a length of four or five inches, contain one-fifth of their dry weight of asparagine. It is an interesting substance to brewers, since it is found in malt rootlets, and can serve as a nitrogenous food for yeast. On

¹ The amino-acids are destitute of acid taste, do not redden litmus, and can be crystallised from alcohol in the presence of ammonia. These phenomena strongly point to the fact that the COOH group of the acid no longer exists as such. Consequently, the

formula $\begin{array}{c} \text{H}_2\text{C}-\text{NH}_3 \\ | \quad | \\ \text{O}=\text{C}-\text{O} \end{array}$ in which nitrogen is quinquivalent, has been suggested to express

the constitution of amino-acetic acid; the analogues would, of course, be represented by formulæ of the same type.

² If, however, the formula, as suggested in the preceding note, is the more correct one, then probably amino-succinic acid has the constitution

being fermented with yeast in the presence of albuminoids, it is converted into ammonium succinate.

Glutamine.—Another amide found in plants is closely allied to asparagine, from which it differs by having a hydrogen atom replaced by methyl, thus:

It is the acid amide of glutamic acid.

Phenylhydrazine.—This substance, which has played such an important part in recent times in the elucidation of the constitution of the members of the sugar group, and which has proved itself of inestimable service in the identification of the various sugars, is a derivative of benzene C_6H_6 . The composition of benzene will strike the reader as being somewhat extraordinary, especially when he is told that it behaves, under most circumstances, like a fully saturated body, indeed, it is difficult to conceive how such a body can be built up with carbon which has four bonds of affinity. The theory first proposed by Kekulé to explain this apparent anomaly assumes that benzene has the following constitution:

in which it will be seen that those bonds of the carbon atoms which are not attached to hydrogen are attached to one another. Several other slightly differing hypotheses have also been propounded to explain the constitution of this compound. When the carbon atoms are arranged in a compound in this way, they are said to form a "closed chain."

If one of the hydrogen atoms in benzene be replaced by the univalent group —NH—NH_2 (which is hydrazine, $\text{H}_2\text{N—NH}_2$, *minus* an atom of hydrogen), the compound phenylhydrazine, $\text{C}_6\text{H}_5\text{—NH—NH}_2$, is obtained.

Phenylhydrazones.—When one molecule of phenylhydrazine combines with one molecule of an aldehyde, ketone, aldose, or ketose, a body termed a "phenylhydrazone," or, more shortly, a "hydrazone," results, thus:

reagent, as in the previous case, form aniline and ammonia. The following equation illustrates the reaction :

It may be observed that, whether we start with the aldose glucose or the ketose fructose, the same body, phenylglucosazone, is obtained. This is a proof of the intimate relation between the aldose and the ketose, of which we shall have to speak further on; and it further shows that the opposite rotatory properties of these two related bodies is dependent on the asymmetric carbon atom marked (α) in each of the formulæ, since this is the only asymmetric atom which suffers change in the foregoing reactions.

The phenylsazones are mostly crystalline bodies sparingly soluble in water, which differ from one another in solubility, melting-point, and optical behaviour, and on account of these properties they have proved of the utmost value in the identification of sugars. Together with the phenylhydrazones they have been found particularly valuable in the discovery of new sugars, several of which have been already isolated by their aid.

Osones.—When phenylglucosazone is heated with strong hydrochloric acid, the phenylhydrazine residues combine with the acid, and a body of the following composition, $\text{CH}_2\text{OH} \cdot 3(\text{CHOH}) \cdot \text{CO} \cdot \text{CHO}$, called “glucosone,” is left. The “osones,” of which this is typical, react with phenylhydrazine in the cold with the re-formation of the osazones. When an osone is subjected to the action of nascent hydrogen, evolved from finely divided zinc and acetic acid, two atoms of hydrogen become attached to the CHO group. Thus when glucosone,

is treated in this way, fructose is obtained.

This reaction is extremely interesting, for by its means we are able to transform an aldose into its corresponding ketose, which, if optically active, always polarises in the opposite direction to that of the aldose from which it was derived.¹

The reverse process, the conversion of a ketose into its corresponding aldose, can also be effected in the following manner. Fructose (levulose), on being treated with sodium amalgam, is partially converted into mannitol, just as is mannose when subjected to the same treatment; and mannitol, on treatment with dilute nitric acid, yields mannose. Consequently we have

¹ Another interesting method of converting an aldose into its corresponding ketose is by reducing its osazone with zinc dust and glacial acetic acid. Taking glucosazone, as an example, the following body is produced: $\text{CH}_2\text{OH} \cdot 3(\text{CHOH}) \cdot \text{CO} \cdot \text{CH}_2\text{NH}_2$, and this, on treatment with nitrous acid in the cold, yields fructose.

here a method of passing from the ketose sugar fructose (lævulose) to the aldose mannose.

Optical Stereoisomerides.—Cases of isomerism are met with among carbon compounds which it is impossible to explain by ordinary formulæ in one plane, and the assumption has been made that these compounds differ by the arrangement of their constituent atoms in space. A formula depicting the spatial relations of the atoms within the molecule is called a stereochemical formula, and isomeric compounds having this relation are known as stereoisomerides. The optical stereoisomerides are a special class of this kind, and consist of compounds which differ in their action towards polarised light. Every compound which in the state of solution rotates the plane of polarised light is capable of existence in at least three isomeric forms; one rotating the plane of polarised light to the right (dextro-rotatory), a second rotating it to an equal extent to the left (lævo-rotatory) and a third, a molecular combination of the other two, without action on polarised light (optically inactive).

The influence exerted on the plane of polarisation when a ray of polarised light is passed through a solution of an "optically active" substance may be compared to the twist which a bullet receives in passing from the breech to the muzzle of a rifle barrel. The cause of the optical activity of compounds was shown by Pasteur to be due to the unsymmetrical character of their molecules. In investigating the isomerism of ordinary (dextro-rotatory) tartaric acid and racemic acid (optically inactive), he noticed that small facets were present in crystals of the former which were absent in the latter; and that these facets were hemihedral, that is to say, they only occurred in half their possible number. In consequence of the presence of these facets the crystals of tartaric acid were unsymmetrical; they gave a nonsuperposable mirror image differing from the actual crystals just as a right hand differs from a left. The crystals of racemic acid, on the other hand, were symmetrical; their mirror image could be made to coincide in space with themselves. Opposite hemihedral crystalline forms, such as those of the crystals of active tartaric acid and their mirror image are termed enantiomorphs. Thinking that the same relations would hold good for the salts of the two acids just mentioned, Pasteur prepared sodium ammonium racemate, and to his surprise found that the crystals of the salt were hemihedral. Further investigation showed, however, that they were in reality a mixture, the hemihedral facets being situated in some cases on the right hand side, in others on the left hand side. The crystals were in fact a mixture of positive and negative forms—the one form being the mirror image of the other. This will be rendered quite plain on reference to the following sketch (Fig. 6) of the crystals of sodium-ammonium salts of dextro- and lævo-tartaric acid respectively.

Having carefully picked out the two enantiomorphous forms, Pasteur made a solution of each, and this led to the important discovery that the right-handed crystals were derived from ordinary dextro-rotatory tartaric acid, whilst the left-handed crystals were derived from a hitherto

unknown acid, having an equal but opposite rotatory power to the dextro-acid.

Pasteur discovered a second method of resolving racemic acid into its optical antipodes. He reasoned that the asymmetries of the tartaric acids would remain unchanged by forming salts with bases, such as ammonia, potash, soda, &c., which are *ex hypothesi* symmetrical, whereas if salts were formed with optically active (asymmetrical) bases their solubilities, crystalline forms, and other properties would differ. If racemic acid were neutralised with, say, a dextro-rotatory base, we should expect a mixture of salts of the dextro- and lævo-tartrates of this base which might be represented as follows :

d-tartrate-*d*-base,
l-tartrate-*d* base,

which, as he remarked, would differ in properties in virtue of a certain difference in asymmetry which they would possess. He employed the

FIG. 6.

asymmetrical base, cinchonine, in his experiments. Having prepared a solution of the acid racemate of this base, he found that the first salt to deposit was the lævo-tartrate, whilst the more soluble dextro-tartrate remained in solution.

Yet a third method of resolving racemic acid into its enantiomorphs—a physiological one—was discovered by Pasteur. It is of special interest to brewers, as it was undoubtedly one of the causes that induced him to turn his attention subsequently to fermentation problems.

Solutions of crude calcium tartrate, such as are obtained in the process of preparing tartaric acid from argol, frequently undergo a kind of fermentation. Pasteur inoculated a solution of ammonium racemate from a fermentation of this kind. The organism thus introduced, which appears to have been a *Penicillium*, set up fermentation in the solution, and this continued up to a certain point and then ceased; it was then found that the solution contained ammonium lævo-tartrate in a state of purity; so that it was evident that the dextro-tartrate alone was susceptible of fermentation by the particular organism present.

Taking the tetra-valency of carbon as a fundamental conception; if we

represent the chemical constitution of a simple compound, such as marsh-gas or methane, by the formula

in which the four valencies are assumed to act in one plane—that of the paper—then on replacing the atoms by others, isomerides would be indicated as possible in instances where experience has not shown the existence of any. Thus there should be two di-substitution derivatives of the types

Attempts to prepare isomeric derivatives of methane have, however, altogether failed, except in the case of the replacement of three of the atoms of hydrogen by three different groups of atoms, in which a special kind of isomerism, which we shall have to consider directly, becomes possible. Whilst, therefore, all known facts go to show that the hydrogen atoms in methane are symmetrically distributed, since an unsymmetrical arrangement in one plane would indicate the existence of even isomeric mono-substitution derivatives, we must seek some other means of representing this.

Pasteur in his lectures on molecular asymmetry in 1860 had expressed himself unable to decide whether the isomerism of enantiomorphous substances was to be explained by assuming that the atoms of the dextro-substance, for example, were grouped in the form of a right-handed spiral, or arranged at the angles of an irregular tetrahedron. The first to suggest the symmetrical arrangements of the four valencies of a carbon atom in space was August Kekulé. This hypothesis was, however, further developed by J. H. van't Hoff, in Holland, and by J. A. Le Bel, in France. Van't Hoff's paper was published in September 1874, and Le Bel's in November of the same year. It was assumed that the four valencies of a carbon atom are directed towards the solid angles of a regular tetrahedron, of which the carbon atom itself occupies the centre. According to this view, methane would be represented by the following symbol :

FIG. 7.

This way of graphically representing the constitution of methane, is in perfect harmony with all the known facts concerning that compound.

Asymmetric Carbon Atom.—No isomeric substitution derivatives of methane should exist, except in cases where each of the four affinities of the carbon atom are combined with a different atom or atomic group, to which attention has already been directed. When this happens, the tetrahedron will become irregular; it will cease to have a plane of symmetry. A compound having the formula CHXYZ ought therefore to exist in two enantiomorphous forms; and this is known to be the case.

As an illustration of this, we may take lactic acid, which may be regarded as methane, in which three atoms of hydrogen have been replaced by the groups CH_3 , COOH , and OH . The two enantiomorphous forms of lactic acid may accordingly be represented as follows (Fig. 8):

FIG. 8.

When a compound contains an asymmetric carbon atom, it is capable of existence in two enantiomorphous forms. Furthermore, it is found that all optically active compounds contain an asymmetric carbon atom, but the converse is not the case, not every substance containing an asymmetric carbon is optically active. Thus the tartaric acids and racemic acid contain two asymmetric carbon atoms, yet, as we have seen, racemic acid is optically inactive.

Bodies like lactic acid, which contain one asymmetric carbon atom, exist in three modifications—dextro, lævo, and inactive. The last modification is obtained by dissolving equal quantities of the right and left bodies, and evaporating the solution. Combination between the two active bodies ensues, the result being the formation of the inactive one. The right and left modifications exhibit, with the exception of slight differences in their crystalline form, the same physical properties, such as the same melting-point, solubility, &c. The inactive form, or “racemic modification,” as it is termed, often, on the contrary, exhibits considerable differences in its physical properties; it can be separated into its active components by appropriate treatment.

Substances, the Molecule of which contains two or more Asymmetric Carbon Atoms.—As the number of the asymmetric carbon atoms in the molecule of a substance increases, so does the number of the stereoisomeric modifications in which it is capable of existing, but the latter increase at a higher rate than the former. Thus four bodies having the structural formula

of tartaric acid are known, the dextro-, lævo-, inactive tartaric acids and racemic acid, all of which contain two asymmetric carbon atoms. The following formulæ show the arrangement of the dextro- and lævo-acids:

In the former both atoms are positive, namely, rotate to the right; in the latter both are negative. The third modification, inactive or meso-tartaric acid, has the following arrangement:

One atom is positive and the other negative; consequently they neutralise or compensate one another, and the result is an inactive body. The compensation in such cases as this is said to be "internal." The fourth modification, racemic acid, is a natural product, but may be prepared artificially by dissolving equal quantities of the dextro- and lævo-tartaric acids, and evaporating the solution. As the two asymmetric atoms of the one acid counterbalance those of the other, the compensation in this case is said to be "external." Racemic acid was the first body in which this peculiar phenomenon was observed, hence the externally compensated form is often called the "racemic" modification. Substances which exist in this modification may, as we have seen, be separated into their optically active components. It is an invariable rule, that whenever a compound containing an asymmetric carbon atom is prepared synthetically in the laboratory it is the inactive racemic substance which is obtained. That is to say, an equal number of molecules of the dextro- and lævo- compounds are simultaneously produced.

The following Table, taken from Tollens's *Handbuch der Kohlehydrate*,¹ shows the possible number of the optical stereoisomeric aldohexoses, which

contain four asymmetric carbon atoms. $\begin{array}{c} \text{H} \\ | \\ \text{C} \\ | \\ \text{OH} \end{array}$ denotes a plus (+) atom, $\begin{array}{c} \text{OH} \\ | \\ \text{C} \\ | \\ \text{H} \end{array}$ a negative (-) atom. Of these sixteen possible members, d-glucose is the ordinary glucose; eleven others have been synthesised by E. Fischer, and four are still unknown:

1. CH ₂ OH	$\begin{array}{c} \text{H} \\ \\ \text{C} \\ \\ \text{OH} \end{array}$	$\begin{array}{c} \text{H} \\ \\ \text{C} \\ \\ \text{OH} \end{array}$	$\begin{array}{c} \text{OH} \\ \\ \text{H} \end{array}$	$\begin{array}{c} \text{H} \\ \\ \text{C} \\ \\ \text{OH} \end{array}$	CHO	d-Glucose (Grape sugar).
2. CH ₂ OH	$\begin{array}{c} \text{OH} \\ \\ \text{C} \\ \\ \text{H} \end{array}$	$\begin{array}{c} \text{OH} \\ \\ \text{C} \\ \\ \text{H} \end{array}$	$\begin{array}{c} \text{H} \\ \\ \text{C} \\ \\ \text{OH} \end{array}$	$\begin{array}{c} \text{OH} \\ \\ \text{C} \\ \\ \text{H} \end{array}$	CHO	l-Glucose.

¹ Vol. ii. p. 14.

3. CH ₂ OH	H C OH	H C OH	OH C H	OH C H	CHO	d-Mannose.
4. CH ₂ OH	OH C H	OH C H	H C OH	H C OH	CHO	l-Mannose.
5. CH ₂ OH	OH C H	H C OH	OH C H	OH C H	CHO	d-Gulose.
6. CH ₂ OH	H C OH	OH C H	H C OH	H C OH	CHO	l-Gulose.
7. CH ₂ OH	H C OH	OH C H	OH C H	H C OH	CHO	d-Galactose.
8. CH ₂ OH	OH C H	H C OH	H C OH	OH C H	CHO	l-Galactose.
9. CH ₂ OH	H C OH	OH C H	OH C H	OH C H	CHO	d-Talose.
10. CH ₂ OH	OH C H	H C OH	H C OH	H C OH	CHO	l-Talose.
11. CH ₂ OH	OH C H	H C OH	OH C H	H C OH	CHO	d-Idose.
12. CH ₂ OH	H C OH	OH C H	H C OH	OH C H	CHO	l-Idose.
13. CH ₂ OH	H C OH	H C OH	H C OH	OH C H	CHO	Unknown.
14. CH ₂ OH	OH C H	OH C H	OH C H	H C OH	CHO	Unknown.
15. CH ₂ OH	H C OH	H C OH	H C OH	H C OH	CHO	Unknown.
16. CH ₂ OH	OH C H	OH C H	OH C H	OH C H	CHO	Unknown.

Of the hexaketoses eight stereoisomerides are possible, and although several have been prepared, definite configuration formulæ have only been assigned to three, as shown in the following table :

CH ₂ OH	H C OH	H C OH	OH C H	CO	CH ₂ OH	d-Fructose (ordinary lævulose of fruit sugar)
CH ₂ OH	OH C H	OH C H	H C OH	CO	CH ₂ OH	l-Fructose
CH ₂ OH	OH C H	H C OH	OH C H	CO	CH ₂ OH	Sorbose

The number of stereoisomeric forms in which a compound is capable of

existing is found by the formula 2^n , n representing the number of asymmetric atoms contained in the molecule. Thus with bodies having the structural formula of glucose, which contains four asymmetric atoms, $n=4$, consequently 2 when raised to its fourth power gives $2 \times 2 \times 2 \times 2 = 16$, the possible number of these stereoisomerides. Similarly, the other aldoses have the following numbers :

Trioses, tetroses, pentoses, hexoses, heptoses, octoses, nonoses, &c.						
2	4	8	16	32	64	128

Constitution of the Sugar Group.—This was for many years a puzzle to chemists, and the views put forward were matters of almost pure conjecture until Kiliani in 1885¹ furnished evidence to show that lævulose (fructose) was a ketonic body which had the following constitution :

Kiliani obtained, by treating lævulose with hydrocyanic acid (HCN), its cyanhydrin, $\text{CH}_2\text{OH} \cdot 3(\text{CHOH}) \cdot \underset{\text{CN}}{\text{COH}} \cdot \text{CH}_2\text{OH}$ (page 33). This body, when hydrolysed with strong hydrochloric acid, yielded the following acid: $\text{CH}_2\text{OH} \cdot 3(\text{CHOH}) \cdot \underset{\text{COOH}}{\text{COH}} \cdot \text{CH}_2\text{OH}$.

On treating this acid with hydriodic acid the following body was produced: $\text{CH}_3 \cdot 3(\text{CH}_2) \cdot \underset{\text{COOH}}{\text{CH}} \cdot \text{CH}$, which is methyl-butyl acetic acid. This indicates that the CO group is situated in the place indicated by the above formula for lævulose, for the added COOH group must undoubtedly attach itself to the CO group. Had it become attached to any other of the groups, an entirely different acid would have resulted.

Turning his attention to glucose, he concluded that the constitution of this body was as follows: $\text{CH}_2\text{OH} \cdot 4(\text{CHOH}) \cdot \text{CHO}$; for on treating this sugar in an exactly similar manner to that in which he had treated lævulose (fructose), the following acid was finally obtained: $\text{CH}_3 \cdot 5(\text{CH}_2) \cdot \text{COOH}$, which is normal heptylic acid; and this distinctly shows that the CHO group, to which the COOH group had attached itself, is at the end of the chain.

The most remarkable work, however, on the constitution of the sugar group is that of Emil Fischer, to be found in volumes xviii. to xxiii. of the *Berichte* of the German Chemical Society, of which the following is a short résumé :

Fischer found that the hexahydric alcohol mannitol,

on treatment with dilute nitric acid, yielded the aldose

mannose, which in many respects resembles glucose. It ferments in contact with beer yeast, reduces Fehling's solution, and rotates the polarised ray to the right, but not so powerfully as glucose. When acted on by bromine it is further oxidised to mannonic acid, $\text{CH}_2\text{OH} \cdot 4(\text{CHOH}) \cdot \text{COOH}$. When a solution of this latter acid is evaporated to a syrup, a molecule of water is

¹ *Ber. d. Deutsch chem. Ges.*, 1885, **18**, 3066.

disengaged, and mannonic acid lactone, $\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}\cdot 2(\text{CHOH})\cdot\text{CO}$ is formed.

Some years before this Kiliani had obtained, by boiling certain gums with dilute sulphuric acid, the pentose sugar arabinose, $\text{CH}_2\text{OH}\cdot 3(\text{CHOH})\cdot\text{CHO}$. On treating this with hydrocyanic acid its cyanhydrin was obtained, and this, on hydrolysis, yielded an acid isomeric with the mannonic acid mentioned above. Fischer, on evaporating the acid obtained from arabinose to a syrup, obtained a lactone similar in every other respect to that of mannonic acid, with the single exception that it was lævo-rotatory, its optical rotatory power being $[\alpha]_D - 54.8^\circ$, whilst the lactone of mannonic acid was dextro-rotatory, $[\alpha]_D + 53.81^\circ$. Making allowance for experimental error, it may be considered that these two bodies possess equal and opposite optical rotatory powers, that they are, in fact, what is known as stereoisomerides, and this was the first instance in which such a relation had been observed amongst the members of the sugar group. When equal weights of the dextro-rotatory and of the lævo-rotatory lactones were dissolved in water, combination ensued and an inactive lactone resulted, which could only be resolved into its optically opposed components by special treatment.

Mannose Group.—This includes the following compounds, which were all that had been produced up to that time.

Nature	Members	d.-Series	r.-Series	l.-Series*
Lactone	Mannonic acid lactone	+	o	—
Acid	Mannonic acid	+	o	—
Aldose	Mannose	+	o	—
Alcohol	Mannitol	+	o	—
Ketose	Lævulose (Fructose)	—	o	+

Here then arose a difficulty with the names of the ketoses, for one of them polarised to the right, another to the left, and the other was inactive. To avoid this difficulty Fischer proposed the name “fructose” for this class of sugars. Also, to signify the series to which any set of members belonged, he suggested appending the letters d-, l-, r-, dextro, lævo- and racemic (inactive), according to the direction in which the aldose member of the series polarised. Ordinary lævulose would then be described as “d.-fructose,” for though it polarises strongly to the left, the aldose of the series to which it belongs polarises to the right.

We have seen that by treatment with a reducing agent (p. 34) it is possible to pass down the series and obtain in turn any member as far as mannitol—that is, it is possible to obtain the ketose from the aldose group through the osazone and osone (p. 39); that by using oxidising agents it is also possible to proceed in the opposite direction, and obtain any member of the series

* The letters d-, l- and r- signify Dextro-, Lævo-, and Racemic respectively. Racemic compounds are optically inactive combinations of two active isomerides analogous with racemic acid (p. 44). They are said to be externally compensated.

member of either the d.-, l.-, or r.- series, all the members belonging to that from mannitol upwards. Consequently, if it were possible to obtain any one particular member, the series could be produced from it; and if any member of the inactive series could be separated into its optically opposed components we should then be in a position to obtain any member whatever of the three series. This has been found possible.

Resolution of the r.- into the d.- and l.- compounds.—This was effected by adding strychnine¹ to a solution of r.-mannonic acid, and thus forming r-strychnine mannonate. This salt, when boiled in alcohol, is resolved into the salts of the d.- and l.-acids; that of the l.-strychnine mannonate, being difficultly soluble in alcohol, falls out of solution. This salt, after the removal of the strychnine from it, yields pure l.-mannonic acid, from which all the members of the l.-series can be formed. The whole of the l.-strychnine mannonate is not, however precipitated from the alcoholic solution, and to obtain the d.-salt in a pure condition, the strychnine is first removed from the solution of the mixed acids and morphine added. From this solution, on long-standing, crystals of pure d.-morphine mannonate separate. After removing the morphine from these, pure d.-mannonic acid is left, from which all the members of the d.-series can be formed.

Glucose.—As previously observed, this sugar very much resembles mannose. It possesses the same structural formula, forms with phenylhydrazine the same osazone, which in turn yields the same osone and ketose (fructose or lævulose). When reduced with sodium amalgam, glucose does not however yield the same alcohol as mannose, but an isomeric one; mannose yields mannitol, glucose yields sorbitol. Their optical activities also differ, that of glucose being much higher than that of mannose; their hydrazones also differ; glucose hydrazone is freely soluble in water, mannose hydrazone is comparatively insoluble. Moreover, gluconic acid forms with calcium a distinct salt, differing in form from the corresponding calcium mannonate. All these facts point to mannose and glucose being stereoisomeres. Fischer considers that the difference in the optical activities of mannose and glucose is due to the arrangement of the groups contiguous to the asymmetric CHOH group which adjoins the CHO group in these two sugars. After various futile attempts to convert mannose into glucose, this was at last effected by heating d.-mannonic acid with quinoline² to 140° C., when a part of the mannonic acid was converted into gluconic acid. After the removal of the quinoline the mixed acids were converted into their respective brucine³ salts. The brucine mannonate, being insoluble, was precipitated, and the brucine

¹ Strychnine is a poisonous alkaloid found in the beans and other parts of the *Strychnos nux vomica*. Morphine is similarly an alkaloid prepared from the inspissated juice of the white poppy (*Papaver albus*).

² Quinoline, C₉H₇N, is a mobile strongly-refracting liquid, first obtained by distilling cinchonine, an alkaloid contained in Peruvian bark, with caustic potash. It is now obtained synthetically by heating together aniline, glycerol, nitro-benzene, and sulphuric acid.

³ Brucine is another poisonous alkaloid, obtained from the *Strychnos nux vomica*.

gluconate remained in solution, from which, after removing the brucine, gluconic acid was left. This, on reduction by sodium amalgam, yields glucose in all respects identical with the ordinary sugar. L- or r-mannonic acid, by similar treatment, yields l- or r-glucose.

Synthetic Sugars.—It has been shown that from any one of the members of the mannitol series the whole of the other members can be formed; hence if we could build up any single member of a series from its elements, it would be possible to produce the whole of the series from it.

So long since as 1861, Butlerow observed that an aqueous solution of formaldehyde, under the influence of certain bases, yielded by condensation a sugar-like body to which he gave the name "methylenitan." Löw, using as the base lead oxide, formed the same or a similar substance, and called it "formose." Fischer obtained, by acting on acrolein bromide with barium hydroxide¹ and afterwards from glycerose $C_3H_6O_3$,² a sugar-like body which he named " α -acrose." He repeated Löw's process, and found formose to be a mixture of various aldose and ketose sugars; for, on treating it with excess of phenylhydrazine acetate, a mixture of osazones was formed, all of which with one exception were soluble in alcohol or ether.³ The insoluble one when treated with hydrochloric acid yielded an osone, which, on reduction with zinc and acetic acid, yielded α -acrose. This body (formed in either of the three ways) afforded, on reduction with sodium amalgam, a body to which he gave the name of " α -acritol," and this resembled mannitol in all other properties but that of optical rotatory power; it was optically inactive. It struck him that α -acritol might be r-mannitol, and α -acrose might be r-fructose; and upon further investigation, this turned out to be the case. Having now obtained two of the members of the mannitol series, it became possible to obtain any other member, and since methylic alcohol, which under proper treatment yields formaldehyde, can be built up from the elements, it follows that the whole of the mannitol series can be similarly synthesised.

Formation of Sugar in Living Plants.—The formation of a sugar from the condensation of formaldehyde is of great interest, since it has been assumed by several chemists and physiologists that this is really the process by which cane sugar, which, according to Brown and Morris,⁴ is the first product of assimilation in plants, is formed. By the agency of the chlorophyll cells of the plant, carbonic acid, H_2CO_3 , is supposed to lose two atoms of oxygen and become formaldehyde, $HCHO$. Six molecules of this body uniting together could form either a molecule of glucose or fructose, and then, by the loss of the elements of water, become cane sugar and starch:

and the ketotriose $CH_2OHCOCH_2OH$, which latter is dihydroxyacetone.

³ Neuberg (*Ber. d. Deutsch. chem. Ges.*, 1902, **35**, 2632, found that a ketopentose was among the products, whilst H. and A. Euler (*ibid.* 1906, **39**, 45) show that this is r-arabinoketose.

⁴ *Journ. Chem. Soc. Trans.*, 1893, **63**, 624.

Usher and Priestly have recently shown that the formation of formaldehyde together with hydrogen peroxide can actually be detected in certain plants, and that the conversion of carbon dioxide and water into these two products appears to be entirely a photolytic action, independent of enzymes and of protoplasm. If this be so, the reaction

is not necessarily a vital process. The hydrogen peroxide is decomposed into water and oxygen by a catalase, which appears to be present in all plants.¹

Disaccharides and Trisaccharides.—These are anhydro-condensation products of the simple sugars into which they are converted on hydrolysis with acid or with certain enzymes. The best known are derived from the hexoses and are given in the following list:

Milk sugar or lactose	$\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$
Maltose	$\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$
Melibiose ²	$\text{C}_{12}\text{H}_{22}\text{O}_{11} + 2\text{H}_2\text{O}$
Sucrose or cane sugar	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$
Trehalose ³	$\text{C}_{12}\text{H}_{22}\text{O}_{11} + 2\text{H}_2\text{O}$
Turanose ⁴	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$
Melezitose ⁵	$\text{C}_{18}\text{H}_{32}\text{O}_{16} + 2\text{H}_2\text{O}$
Melitriose ⁶ (Raffinose)	$\text{C}_{18}\text{H}_{32}\text{O}_{16} + 5\text{H}_2\text{O}$
Stachyose ⁷	$\text{C}_{18}\text{H}_{32}\text{O}_{16} + 3\text{H}_2\text{O}$

E. Fischer regards these di- and tri-saccharides as having the constitution of glucosides, and he has thrown much light on their constitution by a study of the methyl and ethyl derivatives of the sugars. Ordinary glucose, when heated for 50 hours at 100° C. with methyl alcohol containing 0.25 per cent. of hydrogen chloride, yields two isomeric methyl derivatives which do not reduce Fehling's solution and therefore do not contain a free carbonyl group.

Very interesting is the manner in which Fischer has elucidated the constitution of two of these sugars, lactose (milk-sugar) and maltose. Milk-

¹ An interesting review of this work was given in Professor Meldola's presidential address to the Chemical Society, 1906.

² This sugar is one of the products of the partial hydrolysis of raffinose, and has recently been obtained in the crystalline state by A. Bau (*Wochen. Brau.*, 1899, 16, 397). The normal rotation calculated on the anhydrous sugar is $[\alpha]_D^{20} 143^\circ$. On hydrolysis it yields d.-fructose and d.-galactose.

³ Obtained from ergot of rye by Wiggers, in 1832, and first confounded with mannitol. Mitscherlich, in 1857, characterised it and gave it the name mycose. Berthelot, who isolated it from the so-called trehala manna which he supposed to originate from the cocoons of a coleopterous larva from Persia, gave it the name trehalose. It has also been obtained from certain fungi. The rotatory power for the anhydrous substance is $[\alpha]_D^{20} 197.3^\circ$ (*Apping*). On hydrolysis it yields exclusively d.-glucose.

⁴ Obtained by the partial hydrolysis of melezitose.

⁵ Occurs in Turkestan manna and in certain honeydew.

⁶ Found in beetroot, barley, &c. On complete hydrolysis it yields d.-galactose d.-glucose, and d.-fructose.

⁷ From the tubers of *Stachys tuberosa*. On hydrolysis it is converted into a mixture of d.-galactose, d.-glucose, and d.-fructose.

sugar was the first body operated on, and the discovery of its constitution served to settle that of maltose.

When milk-sugar is heated with phenylhydrazine acetate, its osazone (lactosazone) is precipitated, and this, when acted upon by hydrochloric acid, yields the osone. If this be boiled with hydrochloric acid, hydrolysis takes place, and a mixture of equal molecules of galactose¹ and glucosone is obtained. If the mixture be again treated with phenylhydrazine acetate, glucosazone is precipitated almost in the cold (since an osone reacts much more readily with phenylhydrazine than does an aldose), and after heating for some time, galactososazone is also precipitated. This shows that the molecule of milk-sugar is built up from a molecule of galactose and a molecule of glucose, and that the CHO group is attached to the glucose molecule, since it is to this the phenylhydrazine first attaches itself. The CHO group of the galactose is evidently modified in the union of the two molecules, and this arrangement may be probably expressed by the following formula :

Thus the two sugar molecules in combining lose a molecule of water :

Maltose, when treated in the same manner, gives evidence of a similar structure, only in this case the two residues it contains are those of glucose molecules. The formula for maltose is the same as that shown above for milk-sugar, the two sugars being isomerides.

Lactobionic and Maltobionic Acids.—Another proof that the constitution of lactose and maltose as given above is correct is obtained by the consideration of the acids which these sugars respectively yield when treated with bromine.

Lactose gives lactobionic acid :

When this is hydrolysed by boiling with dilute hydrochloric acid, galactose and gluconic acid result :

When maltose is similarly treated with bromine, maltobionic acid is formed, and this, on treatment with hydrochloric acid, yields a molecule of glucose and one of gluconic acid.

¹ A sugar having the same structural formula as glucose, in fact a stereoisomeride of it.

To the disaccharides also belongs cane sugar, $C_{12}H_{22}O_{11}$, which on hydrolysis is converted into equal molecules of glucose and fructose :

Cane sugar does not reduce Fehling's solution, nor does it form a compound with phenylhydrazine. Its constitution is not known with absolute certainty. E. Fischer has suggested the following probable structural formula for this sugar :

Manno-heptose.—This sugar, which has the following structural formula, $CH_2OH \cdot 5(CHOH) \cdot CHO$, has been obtained synthetically by E. Fischer by treating mannose with hydrocyanic acid, when its cyanhydrin is formed, which, on being hydrolysed, yields heptonic acid. The acid having been obtained, all the members of the heptose series can be formed from it. Manno-octose and manno-nonose have been obtained in a similar manner ; the former from the heptose, the latter from the octose.

Pentose Sugars.—A number of these sugars are known ; the aldose members have the constitutional formula $CH_2OH \cdot (CHOH)_3 \cdot CHO$. As with the hexoses a definite number of optical stereoisomerides can exist, in this case eight, and of these six are known. They can be artificially prepared from the hexoses by oxidation, d.-glucose being transformed into ordinary l.-xylose, and d.-galactose into ordinary l.-arabinose. All of them reduce Fehling's solution, and the d.- and l.- varieties possess the power of rotating polarised light. They form compounds with phenylhydrazine, and when heated with strong hydrochloric acid yield half their weight of furfural, thus :

Only two of these sugars, arabinose and xylose, are of interest to the brewer :

Pentosans.—These may be regarded as condensed pentoses, a number of which exist in the lignified walls of the cells of all vegetable tissues ; hence they are present on the husk of barley and malt. They are slightly soluble in water, and on hydrolysis with dilute mineral acids yield pentose sugars. Their empirical formula is probably $C_5H_8O_4$. When treated with strong boiling hydrochloric acid they yield about half their weight of furfural, the pentosans being probably first converted into the corresponding pentoses. They do not appear to be direct products of assimilation, but rather to be derived from pre-existent hexoses.

Ultimate destiny of the Pentosans in Brewing Operations.—Tollens and Glaubitz¹ found that barley and malt practically contained the same amounts

¹ *Jour. f. Landwirthschaft*, 1895, 106.

of these bodies, hence they are apparently unaffected by respiration during the growing process. From a quarter to a third of the quantity of pentosans contained in the malt passed into the wort, probably with partial conversion into pentoses. Out of every 100 parts of the pentoses obtained by boiling brewers' grains with dilute sulphuric acid, although some 73 parts disappeared when fermented with ordinary yeast, only 17 parts of alcohol were formed. Further examination showed that much acid was produced during the fermentation, acetic and lactic acids being identified. With a pure yeast much less acid was produced. On fermenting a solution of arabinose with Hansen's pure yeast, that sugar remained unattacked; but under the influence of an acid-producing bacterium 20.1 per cent. of this sugar fermented, the yield being 3.12 per cent. of alcohol, 4.23 per cent. of volatile acid (reckoned as acetic), and 1.10 per cent. of non-volatile acid (reckoned as lactic). Hence arabinose does not appear to be attacked by yeast, but is readily broken down by bacteria. The furfural-yielding bodies of barley and malt, other than those which yield arabinose and xylose on hydrolysis, apparently behave to some extent like the ordinary hexoses, and unlike the two well-known pentoses just referred to. Thus they are more susceptible of alcoholic fermentation.¹

Estimation of Sugar by Fehling's Solution.—Most of the sugars, in common with other bodies of an aldehydic nature, exhibit (under certain conditions) a strong affinity for oxygen. In virtue of this property, they are able to reduce the oxides of many metals (such as silver, gold, platinum, mercury, and bismuth, when present in alkaline solution) to the metallic form. The metal is precipitated in most cases as a fine powder, in others in the form of a metallic mirror clinging to the sides of the vessel. When an alkaline solution of a cupric salt is acted upon by a reducing sugar, the action does not proceed so far as to withdraw the whole of the oxygen from the cupric oxide; only half of it is removed, the cupric oxide, CuO , being converted into cuprous oxide, Cu_2O . When a solution of glucose is added to a dilute solution of cupric sulphate, and the mixture is made strongly alkaline with sodium hydroxide, there separates out, at the ordinary temperature slowly, but on heating almost immediately, a yellowish red precipitate of cuprous hydroxide, $\text{Cu}_2(\text{HO})_2$, which gradually loses a molecule of combined water and passes into cuprous oxide, which has a bright red colour. This reaction was first used by Trommer as a qualitative test for sugar. It was shown by Barreswil, in 1846, that a solution containing cupric sulphate, potassium or sodium tartrate, and a caustic alkali, will, if these bodies are mixed in proper proportions, remain unaltered on boiling, but that if to the boiling solution a trace of a reducing sugar be added, then an immediate precipitation of cuprous oxide takes place. He also pointed out that this reaction could be utilised for the quantitative estimation of sugar. Some time afterwards Fehling devoted much attention to the subject of sugar analysis, and essentially improved the solution originally proposed by Barreswil. The blue liquid known as "Fehling's solution," is in

¹ Tollens, *Journ. Fed. Inst. Brewing*, 1898, 4, 452.

common use at the present day; it has the following composition: Pure crystallised copper sulphate (34.639 grammes); sodium and potassium tartrate (Rochelle salt) (173 grammes); caustic soda (71 grammes). These salts are dissolved separately in distilled water, their solutions mixed, and the volume of the mixture made up to 1000 c.c.¹ The solution may be used volumetrically, 10 c.c. of the Fehling's solution being added to 40 c.c. of water, the whole brought to the boil, and, while boiling, a dilute solution containing between 0.5 and 1.0 of sugar per 100 c.c. slowly added from a burette until the blue colour of the mixture was entirely discharged. The quantity of grape-sugar required to decolorise 10 c.c. of Fehling's solution, when used in this way, is about 0.05 gramme, but the exact titre of the solution must be experimentally determined, and then the amount of this sugar contained in any solution can be found by a simple calculation.

It was shown by Neubauer² that one equivalent of grape-sugar (180) reduces ten equivalents of cupric oxide (397); but Soxhlet, who subsequently made an exhaustive examination of the subject, found that this was only true when the Fehling's solution was used in the way specified above, and that any change in the dilution of the Fehling's solution, or in the strength of the sugar solution, altered the proportions between the amount of cupric oxide reduced and the quantity of sugar as given by Neubauer. The result of Soxhlet's investigations was an improvement in the volumetric method of sugar estimation.

Quite recently Ling and Rendle have increased the accuracy of the volumetric process of determining sugars considerably by suggesting a new indicator, ferrous thiocyanate, which is prepared in the following manner: 1 gramme of ferrous ammonium sulphate and 1 gramme of ammonium thiocyanate are dissolved in 10 c.c. of water at a temperature of 45–50° C., and the solution immediately cooled; 5 c.c. of concentrated hydrochloric acid are added and the slight reddish colour due to the presence of ferric salt is discharged by the addition of a trace of zinc dust; the indicator becomes red after a few hours by absorption of air; it can be decolorised again by more zinc dust; but its delicacy is impaired after it has been decolorised several times. For the determination of sugars the authors use the Fehling's solution undiluted: 10 c.c. of freshly-mixed Fehling's solution are raised to the boiling point in a flask, and the sugar solution, the concentration of which should be so adjusted that 20–30 c.c. correspond to 10 c.c. of Fehling's solution, is run into the boiling liquid in small amounts, the mixture being boiled after each addition. Towards the end of the titration, drops of the liquid are withdrawn by means of a glass rod, and rapidly brought in contact with drops of the indicator previously placed on a porcelain tile; when all the cupric salt is reduced, the mixture of the two drops ceases to produce a red coloration.³

Working in this way the relative amounts of anhydrous invert sugar,

¹ It is customary to prepare separate solutions of copper sulphate and of alkaline Rochelle salt, and to mix equal volumes of these immediately before use.

² *Archiv der Pharm.*, ii. 72, 278.

³ *Analyst*, 1905, 30, 182.

dextrose and maltose required to reduce a fixed volume of Fehling's solution are in the ratio :

$$100 : 96 : 161$$

But Fehling's solution can be used in an entirely different manner. In this the dilute solution of sugar is added to an excess of boiling Fehling's solution, the precipitated cuprous oxide filtered off, washed, and weighed, either as such, or after conversion into cupric oxide, or after reduction to metallic copper. The first of these (weighing as cuprous oxide) requires special precautions, as the oxide is very liable to absorb oxygen during drying, and increase in weight.¹ The second has been much used, and when performed under certain fixed conditions, such as those laid down by O'Sullivan,² is said to yield results of considerable accuracy. But certain disadvantages attend this and all similar processes which necessitate the employment of a filter-paper. Cuprous oxide comes down in a state of such exceedingly fine division that it is extremely difficult to obtain a filter-paper which will retain the precipitate completely; consequently, a small portion almost invariably passes through the filter and is lost. Filter-paper has also a great attraction for copper salts, which cling so tenaciously to it that they cannot be removed perfectly by washing, and this apparently increases the weight of the precipitate obtained.

In order to avoid these sources of error, it was proposed by Soxhlet to use an asbestos filter, the cuprous oxide being reduced to metallic copper. The asbestos layer is placed in a filter-tube which is so constructed as to permit the precipitate to be readily reduced in a current of hydrogen afterwards.

Tables for Sugar Determinations.—As has been stated previously, the concentration of the solutions used has a marked influence on the relation between the amount of sugar and the amount of cuprous oxide reduced. In order to obviate this source of error, it has been proposed to use always the same quantity of Fehling's solution (60 c.c. undiluted), and the same quantity (25 c.c.) of a sugar solution containing not more than 1 per cent. of sugar, then to ascertain from a table the amount of sugar which corresponds to the quantity of copper obtained. Such a table was constructed by Allihn,³ showing the amount of glucose equivalent to any amount of copper from 0.01 to 0.463 gramme obtained under these specified conditions. In making an estimation, 60 c.c. of undiluted Fehling's solution are placed in a beaker of about 300 c.c. capacity, brought to the boil, 25 c.c. of the sugar solution (which must not contain more than 1 per cent. of sugar) added from a pipette, and the whole allowed to boil up for four minutes. The precipitate is collected, reduced, and weighed in the Soxhlet filter-tube mentioned above. The quantity of glucose contained in the 25 c.c. of sugar solution is found by inspecting the table; for instance, if the reduced copper weighed 0.364 gramme, the amount of glucose would be 0.1923 gramme. Meissl has given a similar table for

¹ O'Sullivan and Stern (*Journ. Chem. Soc. Trans.*, 1896, 1692); Brown, Morris and Millar (*Ibid.*, 1897, 94 and 275); Ling and Davis (*Journ. Fed. Inst. Brew.*, 1902, 8, 477).

² *Journal of the Chemical Society*, 1876, ii. 131.

³ Fresenius, *Quant. Analysis*, ii. 507.

invert sugar, and Wein another for maltose.¹ These have been collated in book form by Dr. Frew.² The latest tables are those of Kjeldahl, and of Brown, Morris, and Millar.³

Cupric Oxide Reducing Power of a Sugar.—It is a matter of great convenience to have a standard to which the reducing powers of the various sugars or mixtures of several of them can be compared. For this standard O'Sullivan proposed⁴ to take the amount of cupric oxide reduced by one gramme of glucose (this being the sugar the reducing power of which was first determined) and call this 100. The quantity of cupric oxide reduced by one gramme of glucose (using O'Sullivan's process) was found to be 2.205 grammes; this he called 100. Later on he suggested that the letter K should stand for the cupric reducing power of glucose; the K of glucose is therefore 100. If one gramme of another sugar is found to reduce only 1.345 grammes of cupric oxide, its cupric reducing power (K) is found by the following sum in simple proportion :

$$2.205 : 1.345 :: 100 : x, \text{ and } x=61$$

The K of such a sugar is therefore 61. In the case of the products of the conversion of starch, it is now more usual to state these reducing powers in terms of maltose. Thus a solution of these bodies which had a reducing power three-quarters that of maltose would be expressed R 75, instead of K 45.75.

The value for the cupric reducing power of a sugar is not a constant one; but varies with the amount of alkali contained in the Fehling's solution, the dilution and other conditions of the experiment.⁵ Brown, Morris and Millar (*loc. cit.*) state that under the conditions of experiment, which they adhere to and which they fully describe, the amount of cupric oxide reduced by one gramme of dextrose varies between 2.321 and 2.558 grammes, the exact amount depending on the extent to which reduction takes place. They also find that Wein's maltose table gives results 5 per cent. too low when the cupric reducing power of maltose is determined by the method of this author. This observation has been confirmed by Ling and Baker.⁶

Solution Factor.—In the course of investigations connected with the carbohydrates it is constantly necessary to ascertain the amount of substance present in a solution. In ordinary chemical practice this is usually effected

¹ It is unfortunate that in these methods the original Fehling's solution is not employed. Each author makes some slight difference in its composition, and this influences the results obtained.

² "Tables for the Quantitative Estimation of the Sugars" (Spon).

³ Kjeldahl, *Compt. rend. Carlsberg*, 1895; Brown, Morris, and Millar, *Journal of the Chemical Society, Trans.*, 1897, **71**, 100 and 281.

⁴ *Journal of the Chemical Society*, 1879, 72 and 275.

⁵ See Heron (*Journ. Fed. Inst. Brewing*, 1896, **2**, 443); O'Sullivan and Stern (*Journ. Chem. Soc. Trans.*, 1896, **69**, 1692); Brown Morris and Millar (*Journ. Chem. Soc. Trans.*, 1897, **71**, 97 and 282).

⁶ *Journ. Chem. Soc. Trans.*, 1897, **71**, 509.

by evaporating a known quantity of the solution to dryness and then weighing the residue. With many of the bodies met with in brewing chemistry, owing partly to their powerful attraction for moisture and partly to their liability to decompose on long-continued heating, it is difficult to obtain a correct result in this way. Consequently, it has become customary to calculate the amount of the carbohydrates present in a solution from its specific gravity. C. O'Sullivan¹ found that when 10 grammes of pure maltose or pure dextrin were dissolved in so much water that the solution measured exactly 100 c.c. at a temperature of 60° F. (15.5° C.), the specific gravity of the solution (10 per cent.) was 1038.5, water being taken at 1000. Assuming that the strengths of such solutions were strictly proportional to their specific gravities, a 1 per cent. solution would have a specific gravity of 1003.85, and solutions containing intermediate quantities would have gravities expressed by intermediate values. Consequently, if each per cent. of either of these bodies raised the specific gravity of a solution by 3.85, it would be a simple matter to ascertain the amount present in solution; it would be only necessary to subtract 1000 from the specific gravity of the solution and divide the figure so obtained by 3.85. Thus 100 c.c. of a solution of maltose of a specific gravity of 1055 would contain $\frac{1055-1000}{3.85} = 14.285$ grammes of that substance. Hence, according to O'Sullivan, the solution factor for maltose or dextrin is 3.85. In course of time it was found that this factor was not quite correct. Brown and Heron² came to the conclusion that 3.85 was too low for maltose, the correct divisor being 3.9314, and O'Sullivan afterwards gave the fresh divisor 3.95 for starch conversion products. It was afterwards found that with solutions of the various carbohydrates the specific gravity of the solution was not strictly proportional to the amount of substance contained in solution, and Brown and Heron³ proposed the use of 3.86 in all cases as a solution divisor; but this is only correct for a 10 per cent. solution of cane-sugar (*i.e.*, 10 grammes in 100 c.c.); which has a specific gravity of 1038.6 at 60° F. Although this factor is not absolutely correct for the products of starch conversion, it was considered that to have one uniform divisor when determining the optical rotating powers and reducing powers of substances in solution would be extremely convenient.

In the majority of cases where bodies connected with brewing have to be examined for cupric reducing power, their amount is most frequently deduced from the specific gravity of their solutions by means of a solution factor. Consequently, it is usual to affix in small characters the solution factor which has been used in any particular case, such as $K_{3.86}$ or $K_{3.85}$. This means that the amount of solid matter in the first instance was estimated by the 3.86 factor, in the second by the 3.85. The absolute reducing power may be readily obtained from the statement of these on any factor, when the true solution factor is known. Thus the reducing power of maltose is

¹ *Journal of the Chemical Society*, 1876, 129.

² *Ibid.* 1879, p. 618.

³ *Ibid.* p. 602.

$K_{3.86} = 61$. If the true solution factor for maltose is 3.9314; then its real reducing power is

$$3.86 : 3.9314 :: 61 : 62.12$$

The solution factors of the various sugars have been worked out with very great care by Brown, Morris and Millar (*loc. cit.*).

Elementary Composition.—To determine the chemical composition of an organic compound, such, for instance, as glucose, a weighed portion of the substance is placed in a tube packed with cupric oxide, and burned in a current of dry oxygen. The carbon of the sugar is oxidised to form carbon dioxide, and the hydrogen to form water. These are collected and weighed; the weight of the carbon dioxide thus formed corresponds to the weight of carbon present in the sugar, and that of the water to the weight of the hydrogen. The amount of these two constituents having been determined, the remainder which is known to be oxygen, is arrived at by difference. For instance, if 300 milligrammes of a substance burnt in this way yielded 440 milligrammes of carbon dioxide and 180 milligrammes of water, from this we should know that it contained in the above weight 120 milligrammes of carbon and 20 milligrammes of hydrogen; and as these add up to 140, the remaining 160 milligrammes must be oxygen. From these data a simple proportion sum gives the percentage composition of the substance; and as has been explained already on p. 27 the empirical formula, in this case CH_2O , can be determined. That is, for each atom of carbon present there are two atoms of hydrogen and one of oxygen. But the question now arises: What is the relative weight of the molecule? If it can be shown to be 30, then the formula of the molecule is CH_2O (formaldehyde); if 60, then its formula is $\text{C}_2\text{H}_4\text{O}_2$ (acetic acid); if 90, then its formula is $\text{C}_3\text{H}_6\text{O}_3$ (lactic acid); if 180, its formula would be $\text{C}_6\text{H}_{12}\text{O}_6$ (glucose).

Determination of Molecular Weight.—The molecular weight of a body may be determined in various ways (see p. 28). But the method chiefly employed in brewing chemistry, where the determinations generally refer to the molecular weights of the carbohydrates, is the cryoscopic or freezing method, first adopted by Raoult. It depends upon the following considerations; All bodies when dissolved in a given solvent lower the freezing-point of the latter, and with the same substance this is proportional to the amount of the substance present in the solution. For instance, if 5 grammes of cane-sugar are dissolved in 100 grammes of water, and the freezing-point of the resulting solution is lowered 0.3°C ., then a solution of 10 grammes of the same sugar in a similar weight of water will have its freezing-point lowered 0.6°C .

But if equal weights of two substances which differ considerably in their molecular weights, as for instance, glucose (molecular weight 180) and cane-sugar (molecular weight 342) are dissolved in equal weights of water, each solution introduced into a test-tube, and the tubes placed in a freezing-mixture, the temperature required by the glucose solution for solidification to

take place will be found lower than that required by the cane-sugar solution. Hence in this case the depression of the freezing-point is not equivalent to the actual weights of the substances present in solution. It has, however, been found to be definitely related to the molecular weights of these substances.¹

If it were possible to dissolve a molecular weight in grammes of any substance, as, for instance, 180 grammes of glucose in 100 grammes of water, the freezing-point of such a solution would have a temperature 10.55° C. lower than the freezing-point of water. As it is impossible to do this, and for other reasons which we cannot enter into here, it is only practical to employ somewhat dilute solutions, the only available method is to obtain the actual molecular weight by a calculation. This is effected in the following manner when water is the solvent employed :

$$\text{Molecular weight} = \frac{19 \times \text{grms. of substance dissolved in 100 grms. of water}}{\text{Observed depression of the freezing-point.}}$$

The figure 19 is what is termed the constant for water.

For instance, 10 grammes of glucose were dissolved in 100 grammes of water, and the depression of the freezing-point of the solution found to be 1.07° C.; then—

$$\frac{19 \times 10}{1.07} = 177.5.$$

This is very close to the real molecular weight of glucose, which is 180. It may be here mentioned that the figures obtained, owing to disturbing influences, are only approximate, and never absolutely exact.

It was found that 5 grammes of cane sugar dissolved in 100 grammes of water gave a depression of 0.285° C.; this, by the above formula, gives a molecular weight of 333, which closely approximates to the true value 342.

Similarly, Brown and Morris² found that 8.6114 grammes of maltodextrin dissolved in 94.71 grammes of water gave depression of 0.180° C. This, when calculated out, gives a molecular weight of 960, and from this and other considerations they considered that the real molecular weight of this substance was 990.

Ordinary and Polarised Light.—In a ray of ordinary light the vibrations of the hypothetical medium ether take place in all directions in a plane perpendicular to the line of propagation of the light; in a ray of plane polarised light the vibrations of the ether particles occur in a single direction only. The conversion of ordinary light into polarised light is accomplished

¹ This law does not hold good for inorganic salts, which behave as though they contained a larger number of molecules than they actually do. This, as explained by the law of Arrhenius, is supposed to depend on what is termed the electrolytic disassociation into the *ions* by the action of the solvent which is assumed to take place in dilute solutions of such salts.

² *Journal of the Chemical Society*, 1889, p. 465.

for practical purposes by passing it through a specially-cut prism of calcspar (Iceland spar), an example of which is the so-called Nicol prism.¹

The Polarimeter and Specific Rotatory Power.—When a beam of polarised light passes through certain substances, it suffers a certain amount of twisting on its axis, or, in other words, it is rotated. The solutions of many substances, such as dextrin, maltose, glucose, &c., possess the power of rotating the polarised ray, and are consequently said to be optically active. Some bodies twist the ray round to the right; these are termed “dextro-rotatory”: others twist it to the left; they are “lævo-rotatory.” All solutions of an optically active substance of the same strength, when traversed by the polarised ray in layers of the same thickness, rotate the ray to the

FIG. 9.—Laurent Half-shadow Polarimeter.

same extent, *i.e.*, each substance has a definite rotatory power. When this is calculated upon a certain definite strength of solution, and upon a layer of such solution of a definite thickness, an expression is found which is termed the “specific rotatory power” or optical activity of the substance. The function of the polarimeter is to determine the amount of rotation which the polarised ray suffers in passing through a layer of the solution of an optically active substance. From this, by taking certain other factors into account, the specific rotatory power of the substance can be found. When the specific rotatory power of a compound present in a solution is known, the polarimeter enables us to estimate its amount.

Laurent Half-Shadow Polarimeter.—There are several varieties of polarimeters, or “polariscopes,” as they are often incorrectly called. The Laurent instrument, which is shown in Fig. 9, is in form something like a telescope. The source of light is placed opposite the end of the instrument,

¹ The description of the nature and properties of polarised light is one of the most intricate branches of the science of optics, hence it is impossible to enter into any details on this subject here. The reader is referred to Landolt's “Optical Rotating Power of Organic Substances” (Second Edition, 1902). A very lucid explanation of the nature of polarised light and of the construction of polarimeters is given by Professor Silvanus Thompson (“On Methods of Polarimetry.” *Trans. of the Laboratory Club*, 1891, 3, pp. 155–176).

J, and at a distance of a few inches from it. In the tube beneath *H* is a prism of Iceland spar, the "polariser," which converts one half of the ordinary light passing through it into polarised light; the other half being reflected to the side and absorbed. At *G* is a thin plate of quartz covering half the field and ground exactly parallel to the optic axis. Its function is to produce the half-shadow effect. Next follows the trough *F*, with light-tight lid, in which the tube filled with the solution under observation is placed. Next comes the tube *E*, which contains another prism of Iceland spar, the "analyser," and the eyepiece *A*. The tube *E* can be rotated on its axis by means of the milled head seen underneath; it carries with it the index *C*, which indicates the observed rotation on the scale of the dial-plate *B*. The light employed with this instrument is monochromatic, and is obtained from a Bunsen burner, in the flame of which a pellet of sodium chloride is supported. The instrument is used in a partly darkened room. The observation tubes are generally made of glass, their ends being ground perfectly true. A brass screw attached to each end enables the tube to be closed water-tight with small circular glass plates, which are retained in position by screw-caps. The lengths of the tubes usually employed are 1, 2, and 2.2 decimetres.¹

In using the instrument, the lamp is lighted and placed in position, and an observation tube filled with water placed in the trough. On looking through the eyepiece with the index at zero, a circle of light will be seen divided into two halves by a black line; the eyepiece is then adjusted until this line is perfectly sharp and distinct. If the instrument is in perfect adjustment, each half of the field will be equally illuminated. If this is not the case, the instrument must be adjusted. If now the observation tube is removed and a two-decimetre tube filled with a 10 per cent. solution of cane sugar inserted, the two halves of the field will be found to be unequally illuminated, but, by turning the index by means of the milled head in the same direction as that in which the hands of a watch go,² a position will be reached when both the fields are again of the same brightness. When this position is reached, the reading of the scale will indicate exactly the amount of twisting or rotation which the polarised beam has undergone in passing through a layer of 10 per cent. cane sugar solution 2 decimetres thick; and from this, as will afterwards be shown, can be deduced the specific rotatory power of cane sugar. The scale on the dial-plate is divided into 360 degrees, and, by means of the vernier attached to the index, can be read to the sixtieth of a degree.

Landolt's Polarimeter.—This form of instrument, shown in Fig. 10, is undoubtedly the best for general purposes. It is so constructed that monochromatic light of any wave length may be used with it. As regards brewing work, however, sodium light would be used, since practically all the

¹ One decimetre = 3.937 inches.

² When the index has to be turned in this direction the angle read is plus (+), when in the opposite direction it is minus (-).

constants of the compounds which are dealt with refer to sodium light. It is furnished with a Lippich polariser with double or triple field, the double field effect being produced by placing a small Nicol prism (called the half-prism), in front of the polarising Nicol, and on one half of it, whilst the triple field effect is produced by two such half-prisms, one on either half of the polarising Nicol, but so that a space is left between them.

For the investigation of substances in a strongly-heated condition, or for the application of very low temperatures, the arrangement shown at G,

FIG. 10.

Fig. 10, may be used. It consists of a rectangular brass box, through which a gold-plated brass tube passes, the projecting ends of which may be closed by glass plates and screw caps. A narrow tube, which is soldered to this observation tube, and which passes through the movable top of the box provides for the expansion or contraction of the optically active substance filled into it. Besides this there are openings in the cover for a thermometer and stirrer. If the box is filled with some substance suitable for a bath, and heated by means of a lamp placed beneath, it is possible to study the rotatory power of compounds at any desired temperature; it is advisable to cover the box with a protecting layer of asbestos, and put up screens to protect other parts of the apparatus as far as possible from high temperature.

If the box is filled with a freezing-mixture for investigations at a low temperature, it is necessary to attach glass cylinders, furnished at the ends with glass plates with plane surfaces, to the screw caps, and put in them a little calcium chloride to prevent the deposition of dew.

Schmidt and Haensch Half-Shadow Quartz Compensating Polarimeter.—

In this instrument, which is illustrated in Fig. 11, the appearance of the circle of light is the same as in the Laurent; the light is, however, obtained from an ordinary gas or paraffin lamp flame, placed at a little distance from the end of the instrument, *N*. The adjustment of the index is made by the milled head *M*, and the scale is read through the eyepiece *K*. The scale is entirely different from that of the Laurent. The instrument is constructed

FIG. 11.—Schmidt and Haensch Half-Shadow Quartz-Compensating Polarimeter.

specially for use in sugar manufactories and refineries; it is therefore furnished with a scale showing percentages of cane sugar directly. The 100 divisions into which the scale is divided can by the aid of a vernier be read to tenths of a division. When the two-decimetre tube is filled with a solution, 100 c.c.¹ of which contain 26 grammes of *pure* cane sugar, the instrument indicates 100 scale divisions. This scale was first used by Ventzke. If an impure sugar is employed for making the solution, and with this the scale reading is 76.5 divisions, then such a sample contains only 76.5 per cent. of cane sugar. Owing to this difference in the scales of the two instruments, it is necessary in order to convert Ventzke divisions into degrees of arc to multiply by a factor. This factor varies, however, according to the optically active substance under investigation. Landolt² gives the following factors for certain of the sugars:

Cane sugar	.	.	.	.	0.3465
Milk sugar	.	.	.	.	0.3452
Glucose	.	.	.	.	0.3448
Invert sugar	.	.	.	.	0.3432

¹ Metric cubic centimetres. If Mohr's cubic centimetres are used the weight of cane sugar must be 26.048 grammes.

² *Ber. d. Deutsch. chem. Ges.*, 1888, 21, 194.

Brown, Morris and Millar¹ have extended these factors, thus:

Cane sugar,	10 % solution	.	.	0.3469
Maltose,	10 % "	.	.	0.3449
"	5 % "	.	.	0.3457
Glucose,	10 % "	.	.	0.3442
"	5 % "	.	.	0.3454
Starch-products,	10 % "	.	.	0.3458
" "	5 % "	.	.	0.3454

Rimbach has proposed the use of the factor 0.344 for all the carbohydrates.

The quartz-compensating instrument only gives absolutely accurate indications with cane sugar and substances whose solutions are of exactly the same dispersive power as quartz; it is fairly accurate also for the carbohydrates, such as glucose, maltose, and dextrin, but with substances such as oil of turpentine its readings would be quite inaccurate. The instrument is more convenient to use than the Laurent, because ordinary white light can be employed, and as the field of view is more strongly illuminated, it is easier to deal with coloured solutions; if absolute accuracy is requisite, the Laurent or better still the Landolt instrument should be employed.

In some of the earlier work, *e.g.*, in that of C. O'Sullivan, the Soleil-Duboscq polarimeter was used, the specific rotatory power being then expressed in degrees $[\alpha]_j$. The use of $[\alpha]_j$ is still retained by some workers. In the Soleil-Duboscq instrument 100 divisions of the scale correspond to 24° of arc, this being the angular rotation experienced by Biot's medium yellow or *jaune moyen* ray (as determined by the transition tint) in traversing a quartz plate of 1 mm. in thickness, cut perpendicular to the optic axis. Brown, Morris and Millar² discuss this very fully. To convert degrees $[\alpha]_D$ into degrees $[\alpha]_j$, the former must be multiplied by the factor 1.1111. These authors state that the expression $[\alpha]_j$ has been used for two rays of different refrangibility which they distinguish as $[\alpha]_j$ Biot, just referred to, and a second $[\alpha]_j$ Montgolfier, having a millimetre-quartz value of 24.5° instead of 24° .

In stating observations made by the polarimeter, it is usual to append the solution factor (*see* p. 56) in those cases where one is employed, as in the statement of reducing powers. Thus $[\alpha]_{D_{3.86}} + 52.8$ would mean that this specific rotatory power had been obtained by employing the 3.86 factor to ascertain the amount of substance present in solution.

Brown, Morris, and Millar³ state that, provided the solution weight of a substance for each degree of concentration is accurately known, they consider the estimation of the solids in solution from the density of the solution is capable of as great, or in most cases of greater, accuracy than by a determination effected in the usual manner by drying, unless special precautions, that are practically impossible in the ordinary routine of analytical work, are taken.

¹ *Journ. Chem. Soc. Trans.*, 1897, **71**, 92.

² *Ibid.* pp. 84-94.

³ *Ibid.* p. 72.

Brown, Morris, and Millar made fresh determinations of the solution weights of a number of the sugars and of various starch transformation products. The last traces of moisture were removed from the substances dealt with by a process devised by Lobry de Bruyn and Van Leent. It consists in placing the sugar or other body in a small flask, which is placed in a water or oil bath, and connected with another small flask containing phosphoric anhydride, a vacuum being maintained in the apparatus during the drying process.¹ From the results thus obtained, the solution factor was determined for various concentrations. They were found, as in the case of cane sugar, not to be directly proportional to the percentages present in solution; they could, however, be expressed in the form of a series of curves. These are given in a table, and by consulting this the proper solution factor for any concentration of any of the sugars given can be found by inspection. The factors for solutions of several of the sugars and starch transformation products at a density of 1055 and at a temperature of 15.5° C., taken from the table, are as follows:

Anhydrous glucose	3.825	Low starch conversion $[\alpha]_D$ 149.7° . . .	3.947
" cane sugar	3.859	Medium " " 173.9° . . .	3.985
" invert sugar	3.883 ²	High " " 188.6° . . .	4.000
" fructose	3.907	Amylin or dextrin	4.206
" maltose	3.916		

In defence of the use of the 3.86 factor for starch conversion products, Brown, Morris and Millar point out that, so far as ascertaining the percentages of the constituents is concerned, the factor employed is a matter of indifference, provided the specific rotatory powers and reducing values of the constituents corresponding with those of the particular divisor taken are used in the calculation. They can be readily calculated into the true amounts as soon as the true factor for the particular starch conversion is known. At the same time, they call attention in a footnote to the fact that this is only strictly correct when the solutions are of approximately the same density, and the constituents possess identically the same divisor; but they do not consider that the error thus introduced is sufficiently large to vitiate their former work, or the conclusions based on it.

When the curves of the divisors for the different grades of starch conversions are examined, it is found that for equal concentrations the divisor for high conversions is greater than that for low—in fact, there appears to be some inverse ratio between it and the amount of apparent maltose present.

It was found that if the mixed products of starch conversions were assumed to consist of dextrin and maltose, and that the maltose, which, according to the amyloïn theory, exists in combination with dextrin, was

¹ Ost (*Chemiker Zeitung*) criticises the above work, and defends his own method for obtaining water-free maltose. He points out that the high temperatures employed by Brown, Morris, and Millar would lead to a slight decomposition of the substances, and that, consequently, all values obtained with such altered bodies must be incorrect.

² The divisor calculated from Brown, Morris, and Millar's results with solutions of glucose and lævulose for a solution of invert sugar of sp. gr. 1055, should be 3.866 and not 3.883 as those authors themselves have given it.

assumed to have the same solution density as free maltose, it became possible to obtain by calculation the divisor for the amylin or dextrin constituent. This was done for various conversions, and the curve thus found is given in the table. Though, as pointed out, it is somewhat improbable that the solution factor for combined and free maltose would be the same, yet it was found that when this dextrin curve was used in conjunction with the maltose curve it was possible to determine, within certain limits of concentration and with a fair amount of accuracy, the solution divisor for the mixed products of any starch conversion brought about by diastase, the apparent maltose percentage being either obtained from the optical rotation or from the cupric reducing power of the solution. A table calculated in this way is given showing the divisors to be employed for solutions of starch conversion products of various concentrations and of different optical rotatory powers and reducing powers. As the optical rotatory power of many substances is influenced by temperature, the temperature of the solution should be noted when an observation is made.

Specific Rotatory Power.—This is the angle indicated by the polarimeter when a layer of the substance one decimetre (3.937 ins.) in thickness is examined in that instrument. For instance, if when the one-decimetre tube is filled with English oil of turpentine, and placed in the Laurent polarimeter, the reading is + 21.5, this number divided by the specific gravity of the turpentine gives directly the “specific rotatory power” of that particular sample of turpentine.

If, on filling a tube of the same length with French oil of turpentine, the angle observed is -40.3° , then the optical rotatory power of that particular sample may be obtained in a similar manner.

When, however, the specific rotatory power of a solid substance, such, for example, as cane sugar, has to be determined, the matter is not quite so simple, as obviously it is impossible to fill the tube with a solid substance like sugar. In such cases a solution of known strength of the body is made with a liquid which is without action on the polarised ray, and the optical rotatory power of the solution ascertained. For instance, when a 10 per cent. solution of pure cane sugar is examined in a tube 10 decimetres long, the angle observed is $+66.5^\circ$. This angle is assumed to express the specific rotatory power or the optical rotatory power of cane sugar, and is expressed thus $[\alpha]_D + 66.5^\circ$. The specific rotatory power of a substance may therefore be described, as O’Sullivan has proposed, as the angle given by a layer of a 10 per cent. solution of that substance 10 decimetres thick. The use of a tube 10 decimetres long, owing to its unwieldiness, would be impracticable, and, moreover, solutions are rarely sufficiently light in colour to permit of the use of a tube of such a length. Consequently, shorter tubes of 1 and 2 decimetres length are almost invariably employed, and the specific rotatory power is obtained by calculation.¹

¹ When the specific rotatory power of a substance is found in this way, it is probably never its real, but only its *apparent* value, since it has been found that solvents, though themselves optically inactive, often have an appreciable effect on the optical rotatory

If, for instance, a 10 per cent. solution of pure sugar be examined in a one-decimetre tube, the angle observed will be 6.65° , and the multiplication of this figure by 10 will give the specific rotatory power of cane sugar. (As 1 decimetre is to 10 decimetres, so is 6.65° to 66.5° .)

If, instead of a 10 per cent. solution, one of 20 per cent. is employed, the angle observed will be twice as large, and will, consequently, have to be halved before being multiplied by 10. Should the two-decimetre tube be employed, then the reading will have to be halved and multiplied by 5.

The specific rotatory power is obtained in any case by the following equation: $[\alpha]_D = \frac{a \ 100}{l \cdot c}$, in which a is the reading of the polarimeter, l the length of the tube employed in decimetres, and c the number of grammes of substance contained in each 100 c.c. of the solution.

For instance, suppose we have a solution containing in every 100 c.c. 12.5 grammes of a body the specific rotatory power of which we wish to determine, and upon examining it in the two-decimetre tube, we obtain a reading of $+41.0^\circ$; then—

$$[\alpha]_D = \frac{+41 \times 100}{2 \times 12.5} = +164^\circ.$$

The specific rotatory power of such a substance is therefore $[\alpha]_D + 164^\circ$.

When the specific rotatory power of a substance is known, the quantity of that substance in solution is readily determined by the polarimeter by the aid of the following formula:

$$c = \frac{a \ 100}{l + [\alpha]_D}$$

c is the number of grammes of the substance in 100 c.c. of solution, a the observed angle, and $[\alpha]_D$, the optical rotatory power of the substance, l being the length of the observation tube in decimetres. For instance, a solution of cane sugar of unknown strength is found to give a reading of $+17.29^\circ$; then—

$$c = \frac{17.29 \times 100}{2 \times 66.5}, \text{ and } c = 13 \text{ grammes}$$

Every 100 c.c. of the solution, therefore, contain 13 grammes of cane sugar.

When the weight and rotatory powers of two bodies in solution are known, the relative proportions of each can easily be found. Suppose that a solution contains 16 grammes per 100 c.c. of a mixture of maltose ($[\alpha]_D + 138^\circ$) and dextrin ($[\alpha]_D + 202^\circ$), and that, on examining the solution in the two-decimetre tube, the angle observed is $+52.842^\circ$, *i.e.*, the solid matter in solution has a specific rotatory power of $[\alpha]_D + 165.2^\circ$; the relative proportions of maltose and dextrin in each gramme of the solid matter are found in the following manner:

From the number found for the specific rotatory power of the mixed power of the substance dissolved in them. This method, though obviously imperfect, is the only one possible, and even then it only indicates what would be the rotatory power of one decimetre of a 100 per cent. solution of cane sugar (if such a thing were possible).

substances subtract that of the constituent having the less rotatory power—in this case it will be 138° —and divide the result by the difference of the rotatory powers of the two bodies, which here is 64.¹ This will be—

$$165.2 - 138 = 27.2, \text{ and } \frac{27.2}{64} = 0.425 \text{ gramme dextrin.}$$

Each gramme of substance in solution consists therefore of 0.425 gramme of dextrin and 0.575 gramme of maltose, consequently the 16 grammes contain 9.2 grammes of maltose and 6.8 grammes of dextrin.

Cellulose.—This substance forms the groundwork of the tissues of every plant from the highest tree to the lowliest fungus. Its chemical composition is expressed by some multiple of the group $C_6H_{10}O_5$, but we are still in ignorance as to what that multiple is. Although one of the most abundant constituents of the vegetable kingdom, it is only met with in the pure state in very young cells, since, as these grow older, other substances are deposited in the cell-walls and in the interior of the cells. This is readily seen in the husk of barley, the cell-walls of which at first consist of cellulose, but which subsequently become incrustated with the tougher and more resistant lignin, in order that they may form a more effectual protection to the more delicate contents of the barleycorn. Cotton-wool or Swedish filter-paper may be cited as examples of nearly pure cellulose. In this condition cellulose is a tasteless and odourless body, insoluble in the ordinary solvents, such as water, alcohol, ether, &c., or by prolonged boiling with dilute acids or alkalis. Cellulose is, however, soluble in a solution of cuprammonium oxide,² from which it is precipitated in an amorphous condition by acids, or by passing a current of carbon dioxide gas through the solution. It is also soluble in a strong solution of zinc chloride in hydrochloric acid.

Cellulose is coloured yellow or brown by a solution of iodine; but if previously treated either with a strong solution of sulphuric acid or of zinc chloride, it is then coloured blue by iodine.

Cellobiose ($C_{12}H_{22}O_{11}$).—When cellulose, in the form of cotton wool or filter paper, is treated with concentrated sulphuric acid and acetic anhydride it is converted into the octacetyl derivative of this biose, and this biose is obtained by hydrolysing the acetyl derivative with alcoholic soda. It does not possess a characteristic taste, but the after flavour is faintly sweet. It reduces Fehling's solution, but is only slightly fermentable, if at all, by yeast. Its specific rotatory power is $[\alpha]_D = 33.7^\circ$; it exhibits birotation. When hydrolysed with acids it yields dextrose and perhaps another sugar.³

When cellulose membranes have attained a certain age they exhibit the

¹ Expressed algebraically, this would be as follows :

$$\begin{array}{rcccl} 202x & + & 138(1-x) & = & 165.2 \\ \text{Rotation of dextrin.} & & \text{Rotation maltose.} & & \text{Rotation of mixture.} \end{array}$$

² Made by placing copper turnings in a vessel partially filled with strong solution of ammonia and permitting free access of air. The liquid gradually assumes a deep blue colour.

³ Skraup and König, *Berichte d. Deutsch. chem. Ges.*, 1901, **34**, 1115.

phenomenon of double refraction. Some of the celluloses, when first treated with somewhat concentrated sulphuric acid, yield glucose on prolonged boiling with a dilute solution of the same acid. These may, therefore, be regarded as polymerised anhydrides of this sugar. Other varieties, when similarly treated, yield mannose, and this is particularly characteristic of what are termed the "reserve celluloses," such, for instance, as those which are contained in large quantity in the ivory nut or the stone of the date. Here cellulose is deposited as a reserve material, just as starch is in other seeds, such, for instance, as in those of the barley plant. For this form of cellulose (which is somewhat more readily soluble in strong alkaline solutions than the other) the name of "hemi-cellulose" has been proposed. Often the cell walls of plants consist of a mixture of these two forms of cellulose, which exist most probably in a state of chemical combination. It has been shown by Cross and Bevan that there are still other modifications of cellulose present in many vegetable tissues, such as those of the barley plant; and these, since they contain a larger percentage of oxygen than the ordinary cellulose, they have named "oxy-celluloses." They are extremely resistant to the action of dilute alkalis. Like the pentose sugars, the oxy-celluloses, when distilled with hydrochloric acid, yield furfural; and, upon hydrolysis with dilute acids (1 per cent. sulphuric acid), are partially converted into a fermentable sugar to which Cross and Bevan assign the following probable constitution, $\text{O}_3\text{H}_5\text{C}_5\begin{smallmatrix} \diagup \text{O} \diagdown \\ \diagdown \text{O} \diagup \end{smallmatrix} \text{CH}_2$; but Tollens¹ expresses grave doubts as to the correctness of this formula. They point to the possibility,² by first boiling spent brewers' grains for two hours with 1 per cent. sulphuric acid solution, and then neutralising the acid with chalk, of obtaining a further portion of fermentable sugar, which might be added to the wort previous to fermentation. At all events, the sugar obtained in this way should be utilisable by the distiller and vinegar-maker.

Hydrocellulose.—A form of cellulose is met with in the delicate parts of young plants which is partially soluble in dilute alkalis, and this has been termed hydrocellulose.

Hemicelluloses.—These, like the hydrocelluloses, are soluble in 2 to 5 per cent. solution of sodium hydroxide. Some of these are hexosans, since, on hydrolysis, they yield glucose, galactose, &c., whilst others are pentosans, for under similar conditions they yield pentose sugars.

The so-called starch cellulose present in starch granules appears to be of a different nature to ordinary cellulose; it probably differs from the other carbohydrate constituents of the granules by containing less of the elements of water in its molecule.

The husk of barley which consists of lignified cellulose ranges from 7.2 to 14.9 per cent. by weight. It may be determined by soaking the barley, and placing it while still under water in the receiver of an air-pump, and

¹ *Journ. Fed. Inst. Brew.*, 1898, 4, 453.

² *Ibid.* 1897, 3, 15.

exhausting the air. This removes the air contained in the barley; and, on again admitting air, the pressure of the atmosphere forces water into the spaces originally occupied with air, the effect being to so loosen the husks that they can be easily removed. They are then dried and weighed.

Lignin.—This constituent of plant tissues, met with in wood and the lignified parts of plants, is distinguished from cellulose by several properties, such as insolubility in cuprammonium oxide solution, in not being coloured blue by iodine and sulphuric acid, etc. When thin sections of a plant tissue containing lignin are treated with phloroglucinol and hydrochloric acid, and examined under the microscope, this constituent is seen to be coloured red. Lignin, when distilled with strong hydrochloric acid, yields furfural in considerable quantity; when boiled with dilute hydrochloric or sulphuric acid it yields either one or both of the pentose sugars arabinose and xylose. Lignin evidently consists partially of pentosans.

Starch or Amylum.—To the brewer this substance is the most important constituent of the barleycorn, of which it forms 60–66 per cent.; indeed the sugar, which by fermentation is to yield alcohol and carbon dioxide, is for the most part derived from starch.

Next to cellulose, starch, which is found in all green plants, is the most abundant material met with in the plant world. It is stored up in considerable quantities in the seeds of many plants, such as the cereals, as a reserve material from which the seedling derives its nourishment during germination.

Characteristics of Starch.—Starch in a state of purity forms a white, tasteless and inodorous powder consisting of small granules. The starch grains from different plants, when examined under the microscope, are found to differ so much in form and size that it is often possible to determine, by a simple microscopical examination, from which plant the starch has been derived. The illustrations on pp. 72 and 73 show this. Though all these starches differ in size and shape, still they exhibit certain similarities in structure. Each has a dark point, centric in some, excentric in others, known as the “hilum.” Round this are seen a series of concentric lines, an appearance caused by the peculiar structure of starch granules, which are built up of layers containing varying amounts of water. The hilum is always rich in water, and each layer alternately contains more and less water, the outside layer being always the poorest in water and richest in substance. As a consequence of the proportion of water increasing from the outside of the granule inwards to the hilum, fissures radiating from the hilum toward the periphery often arise as the granule becomes dry. By soaking starch granules in alcohol, which entirely deprives them of water, all appearance of stratification disappears, but the lines reappear if the granules are moistened with water. If they are treated with dilute alkalies or acids, or with a weak solution of chromic acid, the appearance of stratification is rendered much more distinct.

Starch is insoluble in the ordinary solvents, such as alcohol, ether, &c.,

as well as in a solution of cuprammonium oxide. The intact grains are completely insoluble in water, and hence starch is readily obtained from seeds, tubers, and other starch-containing organs. These are crushed or ground and washed with water on a sieve; the starch granules pass through the sieve along with the water, leaving the coarser portions of the tissues behind. On standing, the starch deposits on the bottom of the vessel placed to receive it and the liquid which passes through the sieve. It is then washed several times with water by decantation, and finally dried at a low temperature. Starch is generally made from rice in England, from potatoes and wheat in Germany, and from maize in Southern Europe and America.

The average specific gravity of air-dried starch is 1.503 to 1.504. Air-dried starch contains from 15 to 20 per cent. of hygroscopic water, the last traces of which it retains with great pertinacity; consequently, it is almost impossible to remove the whole of the moisture by heat alone without at the same time causing a chemical alteration in the substance itself. To avoid this it has been proposed by Dafert¹ to remove the hygroscopic water by drying starch in a vacuum at 100° C. (212° F.). Absolutely dry starch attracts moisture with such avidity, that when moistened with water a perceptible rise of temperature takes place. This shows that the last portions of water are in a state of chemical combination.

Formation of Starch.—The starch granules are deposited in certain cells in the plant tissue known as “starch-formers” or “amyloplasts.” According to the theory propounded a long time since by Nägeli, starch granules do not grow like a crystal from within outwards (by apposition), but from without to within (by intussusception). A layer of starch is first deposited round the inner surface of the starch-forming cell, which is filled with a solution of the substance (probably sugar) from which the starch is derived. Another layer is deposited inside this, and so on until the cell is completely filled. According to this view, the outside layer of starch is the oldest and poorest in water; the portions about the hilum are the newest and richest in water. Attempts have been made by Schimper and Meyer² to disprove this theory, and to show that the starch granules grow by apposition; but the older theory (intussusception) of Nägeli appears to explain the whole of the phenomena observed in the more satisfactory manner, and is the one which has found the most general acceptance.

Microscopical Characters of Starch Granules.—In microscopical appearance barley-starch (Fig. 12, *a*) consists chiefly of two sets of granules, one very much larger than the other, also a few of an intermediate size. The larger granules are circular in shape, and when viewed from their sides have the appearance of bi-convex lenses; the smaller granules are either spherical or polygonal. The hilum is centric, but is seldom well marked. The concentric markings are very indistinct, unless brought out by some reagent, such as chromic acid. The smaller granules are entirely unstratified. The

¹ *Journ. f. prakt. Chem.*, 73, 51.

² *Botan. Zeit.*, 1881, pp. 185 and 841.

large grains vary in diameter from 10.8 to 32.8μ , the majority measuring 20.3μ . The lesser ones are from 1.6 to 6.4μ in width, the most frequent size met with being 4.6μ .¹

Wheat-starch (Fig. 12, *b*) very much resembles barley-starch in appearance; the small granules of wheat are, however, smaller than those of barley, and the large granules show the concentric markings even less distinctly. The large granules vary in diameter from 11.1 to 41μ , and the smaller from 2.2 to 8.2μ .

Rice-starch (Fig. 12, *c*) consists of two kinds of granules, simple and

FIG. 12.—Starch Granules from—(a) Barley; (b) Wheat; (c) Rice; (d) Maize.

compound. Both are polygonal in shape. A large polygonal or a star-shaped hollow takes the place of the hilum. The compound grains vary in diameter from 18 to 36μ , the simple grains from 3 to 7μ .

Maize-starch (Fig. 12, *d*) also consists of simple and compound granules. In the exterior horny portion of the corn the granules are polyhedral and compound, but the interior and more mealy portions of the grain consist almost exclusively of simple granules, only a few really compound granules being met with in this position. The simple granules are spherical or elliptical; the compound granules, and also the small grains contained within them, are polygonal. The majority of the granules possess a hilum, or in its place a star-shaped hollow. The compound granules have a diameter of about 47μ , the simple vary from 7.2 to 32.5μ in diameter, the average being 20μ .

In *potato-starch* (Fig. 13, *a*, *b*) the large and mature granules are egg-shaped, the smaller and more immature spherical. The larger ones have an excentric hilum, around which the excentric markings are very distinct; the small granules exhibit no signs of stratification. The granules of this starch vary in diameter from 60 to 100μ , their most frequent size being 70μ . Potato-starch is known commercially as “farina.” Of this there are two kinds, *prima* and *secunda*, distinguished by the size of the respective granules. The *prima* consists of large granules varying from 21 to 33μ in diameter; the *secunda* of granules from 12.5 to 21μ in diameter.

Sago-starch (Fig. 13, *c*) is somewhat elliptical in form, the hilum often appearing as a fissure situated in the long diameter of the granule. The cells have a tendency to split in two through their short diameter, thus producing

¹ The micromillimetre, which equals the one-thousandth part of a millimetre, is an exceedingly convenient unit for microscopical measurements, and is denoted by the Greek letter μ . It is equal to the $\frac{1}{25,000}$ of an inch.

muller-shaped granules. As met with in commerce, these characters are much modified by the heat and moisture used in the process which this starch undergoes in being made into grains.

Composition of Starch.—According to Nägeli, starch chiefly consists of two bodies, granulose and starch cellulose. It also contains minute quantities of fat, nitrogenous bodies, and ash. The granulose of the starch grains is easily dissolved by several reagents which do not affect the cellulose, con-

FIG. 13.

sequently a separation of the two can be readily effected. If starch grains are digested with a little fresh saliva at 35° to 55° C. (95° to 131° F.), or treated at 60° C. (140° F.) with a saturated solution of common salt containing 1 per cent. of hydrochloric acid, the granulose gradually dissolves away, and a perfect skeleton of the cellulose is left behind which exactly resembles in form the original starch cell. The substance thus obtained is coloured from yellow to brown by iodine; if previously treated with sulphuric acid or chloride of zinc, iodine then gives a blue coloration.

Arthur Meyer puts forward the view that starch granules consist of two modifications of amylose besides, to some extent, their hydrolytic products. The one is the anhydro form of the other; it is α -amylose and is the starch cellulose of Nägeli. The other is β -amylose (soluble starch). It is highly probable that starch granules consist of more substances than these two, the differences between them being merely in the degree of hydration. Such a view probably explains partially the differences between the starch granules of different origin.

Dragendorff¹ found, by treating various starches with 1 per cent. of hydrochloric acid at 60° C. (140° F.), amounts of starch cellulose varying from 2 to 6 per cent. in the different starches; and similar results were obtained by Nägeli.² If the action of the acid be continued too long, some of the cellulose dissolves, since it is not altogether unattacked by dilute acids. The outside thick envelopes are the most resistant.

¹ *Journ. f. Landwirthsch.*, 1862, p. 211.

² *Beit. z. Kennt. d. Stärke*, 1874.

According to the investigations of Sostegni,¹ starch cellulose consists of two substances, one of which is a fat. This portion, which constitutes about one-fifth of the whole substance, can be extracted by ether. It is a pure white crystalline body, which melts at 47° to 48° C. (117° to 119° F.). The remaining portion of the starch cellulose is soluble in a 2 per cent. solution of caustic potash, and from this solution it is precipitated as an amorphous substance on the addition of an acid. If heated for some time with a dilute mineral acid, it dissolves and yields glucose.

Granulose.—According to the views of the older investigators, the greater portion of the starch cell, and that which is coloured blue by iodine, consists of granulose. When the structure of the starch cell has been destroyed, either by heat or mechanical force, the granulose can be dissolved out by cold water. Berzelius,² by triturating starch in a mortar with cold water, was able to dissolve everything but the cellulose portion. Nägeli states that he has seen the granulose dissolve under the microscope in sections cut through starch grains.

Notwithstanding these statements, it is questionable if granulose forms a true solution. When a so-called solution is filtered through filter-paper, the granulose passes through, and its presence can be detected in the filtrate by the deep blue colour given by iodine, though no trace of suspended particles can be seen. If, however, a diffusion experiment be made with parchment paper, no granulose passes through the membrane. Hence it is fair to conclude that the granulose is not in a state of true solution, but is merely divided into exceedingly fine particles, which are diffused through the liquid. These particles, though sufficiently small to be able to pass through filter-paper, are not small enough to pass through a membrane by osmosis.

Soluble starch prepared by Lintner's method in its aqueous solution has specific rotatory power of about $[\alpha]_D + 202^\circ$ and no reducing power; it is precipitated from such solutions by alcohol and tannin.

Behaviour of Starch with Iodine.—Starch granules or solutions of granulose are coloured an intense blue on the addition of a dilute solution of iodine. This reaction, which was discovered by Gaultier de Claubry in 1814, is extremely delicate, and is frequently used as a test for the recognition of minute traces of starch or of iodine. It is much influenced by temperature; the lower the temperature the more sensitive is the reaction; at or near the boiling-point of water the coloration does not appear at all. It does not show itself so readily, nor is the colour so pure, in the presence of certain other bodies, such as tannin, malt extract, beer yeast, &c. Whether the blue substance formed by iodine and granulose is a true chemical compound or not has not been definitely settled.

The colour produced by iodine and starch grains is not always a pure blue but varies with the nature of the starch. Thus, potato-starch gives a deep

¹ *Central f. Agriculturchem.*, xv. 638.

² *Jahresber. f. Chemie*, 1831, p. 201.

blue, whilst with wheat-starch the colour produced inclines somewhat to violet. This may perhaps be accounted for by a larger amount of the cellulose, which is coloured brown by iodine, being contained in some of the starches.

Two starches have been met with by Dafert and Kreusler,¹ the one in a species of rice, the other in a kind of millet, which are coloured from red to brown by iodine.

As seen from the above observations starch granules are by no means homogeneous. Even the proximate constituents of the granules are not chemical entities. Brown and Heron have indicated the existence of two forms of starch cellulose, one soluble in boiling water and the other soluble in potash, the solution after acidification giving a blue coloration with iodine. A. Fernbach states that the large and small granules of potato starch have a different composition; both contain phosphorus, the smaller granules more than the larger. A. Fernbach and J. Wolff² have shown that barley and other raw grains, as well as malt, contain an enzyme, *amyl-coagulase*, which produces a coagulum when allowed to act on starch solution. The coagulum is a mixture of substances containing amylocellulose, which latter is now recognised as reverted starch. The starch isolated from green peas, according to Fernbach and Wolff, has properties closely analogous to those of amylocellulose or coagulated potato starch. The amylocellulose (starch cellulose of Brown and Heron), which deposits from starch paste (the quantity formed being the greater the higher the concentration of the paste), is itself a mixture. E. Roux³ has shown that the formation of amylocellulose from starch paste is a reversible reaction between 0° and 150° C. At the latter temperature, in presence of excess of water, it liquefies and undergoes a degradation into a simpler form which is coloured blue by iodine. By incomplete degradation of amylocellulose, Roux had produced artificial starch granules. These granules, when acted on by malt diastase at 56° C., yield 98 per cent. of apparent maltose.

L. Maquenne and E. Roux⁴ state that ordinary starch paste contains about 80 per cent. of a substance which is identical with artificial starch, *i.e.*, amylocellulose. This is partly soluble in boiling water and completely so in water at 150° C.; its solutions are devoid of gelatinising power and give a blue coloration with iodine. Acted on by diastase, it is completely converted into maltose. These authors term this substance in the pure state amylose. The remaining 25 per cent. of ordinary starch consists, according to them, of *amylpectin*, a mucilaginous substance to which the gelatinous nature of starch paste is due. It gives no colour with iodine and yields no reducing sugar under the action of diastase. Amylocellulose can exist in a solid or liquid form which are intercon-

¹ *Landwirth. Jahrbuch*, 13, 767; 14, 831.

² *Journ. Inst. Brewing*, 1904, 10, 216.

³ *Comptes rend. de l'Acad. des Sciences*, 1905 140, 440, 943, 1261.

⁴ *Ibid.*, 1303.

vertible, by heating under pressure or by cooling the concentrated solution (reversion). In the starch granule it occurs in a state resembling the liquid modification capable of dissolving in boiling water and reacting with diastase and iodine; it is suggested that it is held there by the amylopectin in a state of solid solution.

Imbibition.—Starch is able to imbibe a certain amount of cold water, which simply swells the granules out to a slight extent without destroying their organised structure.

Gelatinisation of Starch.—If starch be mixed with cold water and the mixture be gradually heated, when a certain temperature (which varies for each different starch) is reached, the granules begin to swell, and when a certain point is reached, their cellulose envelopes burst. As the temperature rises, more and more of the contents of the granules escape, until at last a gelatinous mass is produced, thinner or thicker in consistence according to the amount of starch present; this is known as “starch-paste.” The granules, when examined under the microscope, are now found to have lost all structural organisation, and this is never afterwards regained. If the paste be dried, a horny mass is left, which, when heated with water, merely swells up, but does not dissolve. When starch-paste is frozen, the mass afterwards thawed and the water pressed away, the starch after drying is left as a loose spongy white mass, which upon being boiled with water merely swells up, and exhibits no tendency to form a gelatinous paste. With gelatinisation the starch granules cease to be doubly refractive. The viscosity of starch-paste varies with the temperature, the same paste being much less viscous when hot than when cold. The temperature at which the starch has been dried has a marked effect upon the viscosity of the paste derived from it. Brown and Heron¹ found that the viscosity of starch-paste made from starch dried at 30° C. (86° F.) was only a fourth of that made from the same starch when dried at 100° C. (212° F.).

If thin warm starch-paste be filtered, the portion passing through the filter is quite clear, and gives a deep blue colour with iodine; no solid matter can be detected in the liquid by a microscopical examination. Märker expresses doubt as to whether the granulose is really in a state of true solution; Brown and Heron hold an opposite opinion, and think that the granulose does go into solution, and that the viscosity of starch-paste is to be attributed to the swollen cellulose. A later view, taken by Moritz and Morris,² is that on boiling starch the granulose is converted into soluble starch.

Whether in a state of true solution or not, starch-paste is quite non-diffusible, and incapable of passing through a porous membrane.

Temperatures at which Gelatinisation Occurs.—The temperature at which gelatinisation commences varies with the different starches, and a higher temperature than this is always required for its completion.

¹ *Journ. Chem. Soc. Trans.*, 1879, 35, 614.

² *Text Book of the Science of Brewing*, p. 95.

The following table¹ gives the gelatinisation temperatures of several of the commoner varieties of starch :

	C.	F.
Potato-starch	65°	149°
Barley-starch	80°	176°
Green-malt-starch	85°	185°
Kilned-malt-starch	80°	176°
Oat-starch	85°	185°
Rye-starch	80°	176°
Wheat-starch	80°	176°
Rice-starch	80°	176°
Maize-starch	75°	167°

Starch entirely free from alkalis or acids when heated with water under pressure to 150° C. (302° F.) is converted into that modification known as "soluble starch." This property is now frequently made use of in the analysis of substances which contain starch.

Action of Acids on Starch.—Starch is gelatinised and dissolved by fuming hydrochloric acid in the cold to a clear solution, which principally contains soluble starch. Dilute acids split up, by hydrolysis, the complex molecule of starch into simpler molecules—dextrins, maltose—the final product being glucose. Hydrochloric acid has the most intense action in this direction, sulphuric acid acts less energetically, and the organic acids, such as oxalic, are much slower and more feeble in their action. Elevation of temperature essentially hastens the hydrolytic action. When starch is allowed to stand for seven or eight days under a 7.5 per cent. solution of hydrochloric acid, it is converted into soluble starch.

Rolfe and Defren² found that there is a fixed relation between the optical rotation and cupric reducing power of starch transformation products yielded under widely different conditions, and which were assumed to be maltose, glucose, and dextrin. Hence, if the one value is known the other can be calculated from it. They also constructed a noteworthy table, showing the percentages of the three bodies for different polarimetric readings, between $[\alpha]_D$ 195° and 53.5° and found these to agree with the composition of the products obtained on the manufacturing scale. Hence they conclude that the same specific rotatory power always represents the same chemical composition in any homogeneous starch conversion by acid. The tint given by the iodine reaction is used in glucose factories to determine the completion of the conversion which varies between $[\alpha]_D = 128^\circ$ to 135° .

Considerable doubt has been expressed as to whether maltose is an intermediate product in the acid hydrolysis of starch. Thus Lintner and Düll, who studied the action of oxalic acid on starch, came to the conclusion that the sugars formed were "isomaltose" and finally glucose. The work of Liñg and Baker, of Brown and Morris, and of Ost showed, however, that the "isomaltose" which Lintner claimed to have separated from the products of the action of

¹ Lintner, *Brauer u. Mälzer Kalendar*, 1880.

² *Journ. American Chem. Soc.*, **18**, p. 869.

diastase on starch is not a homogeneous compound but a mixture—Dierssen confirmed Lintner and Düll as regards the production of “isomaltose” by the acid hydrolysis of starch, whilst Grütters found that the supposed “isomaltose” formed under these circumstances is a mixture of maltose and maltodextrin- γ ($C_{12}H_{20}O_{10}$)₂, H_2O , isomeric with the maltodextrin- β of Ling and Baker. Ost confirmed this result and succeeded in obtaining maltose in crystals from the mixture having the constants of “isomaltose.”

Constitution of the Starch Molecule.—Much doubt exists even as to the size of the starch molecule. Musculus and Gruber considered that it contained the group ($C_{12}H_{20}O_{10}$) five or six times; Brown and Heron assumed it to be $10(C_{12}H_{20}O_{10})$; and Brown and Morris $5(C_{12}H_{20}O_{10})_5$.

With reference to its constitution we are as yet entirely in the dark. An important step towards the elucidation of this enigma was made by Scheibler and Mittelmeier,¹ who showed that the dextrans possess free carbonyl (CO) groups, and hence reduce Fehling's solution. They look upon starch granulose as consisting of an unknown number of glucose residues, united by a number of monocarbonyl groups and one dicarbonyl group, since, like cane sugar, it has no reducing power. Of the many conceivable formulæ the following two are put forward as the most probable:

R indicates a glucose residue, < a monocarbonyl and < > a dicarbonyl union.

As the formulæ show, when cleavage takes place at a dicarbonyl union, the resulting molecules have each a free CHO group, and each, therefore, possesses reducing powers. If it be assumed that, when starch is hydrolysed, dextrin and maltose are formed simultaneously, then the first phase of the action may be explained according to one or other of the following formulæ, in which < denotes a free CHO group:

or

In the first case, besides maltose, a dextrin with a free CHO group, and consequently possessing reducing power, would be obtained; in the second, maltose and a dextrin without reducing power.

In a third paper the authors² considered that they had not only effectually disposed of the hypothesis of Brown and Morris of 1889, but had also placed the question of the chemical constitution of starch in an entirely new light.

¹ *Berichte d. Deuts. chem. Ges.*, **23**, 3060.

² *Ibid.* 1893, p. 2930.

Taking into account the views of E. Fischer on the structural formulæ of the disaccharides maltose and lactose, they regard that of cane sugar as $C_6H_{11}O_5 \cdot O \cdot C_6H_{11}O_5$. Since they consider starch to have a similar constitution it would be expressed by the formula $C_6H_{11}O_5 \cdot O \cdot (R)_x \cdot C_6H_{11}O_5$, in which R_x represents an unknown number of glucose residues. As the hydrolysis of cane sugar may be represented by the equation—

so may that of starch by—

Applying their symbols to the various linkings, the formula for cane sugar becomes $C_6H_{11}O_5 < O > C_6H_{11}O_5$, and that of starch,

and maltose $C_6H_{11}O_5 < O \cdot C_6H_{11}O_5 <$. As they had already shown that the dextrins possessed all the qualitative properties of maltose, their general constitution may be expressed by the following formula :

the number of the glucose residues R , varying as the dextrins themselves vary.

Scheibler and Mittelmeier do not consider that the breaking down of starch by hydrolysis takes place according to Musculus's theory, but believe it to occur in a manner similar to that in which raffinose

does. This sugar breaks down in two distinct phases ; in the first the mono-carbonyl link (marked with an asterisk) gives way, a molecule of fructose being split off, leaving a residue of melibiose ; in the second phase the dicarbonyl link gives way, and the melibiose breaks down into glucose and galactose.

Brown and Millar,¹ in a paper to which further reference is made (*see* p. 175), direct attention to the resting stage in the reaction between diastase and starch paste, which was first observed by Brown and Heron, and Brown and Morris, and designated the No. 8 equation. It is thus represented :

This equation assumes that the dextrin (stable dextrin) is a substance without cupric reducing power with an empirical formula $nC_{12}H_{20}O_{10}$; but Brown and Millar show that it has a reducing power of R 5.7–5.9, and they assume that its formula cannot be expressed in a simpler form than by $40C_6H_{10}O_5 + H_2O$. The C_6 formula is chosen on account of the fact that the dextrin breaks up into dextrose as well as maltose when treated with diastase. To bring the above equation into line with these facts it requires modifying

¹ *Journ. Chem. Soc. Trans.*, 1899, 333.

in such a manner as to show the interaction of $\frac{1}{10}$ molecule H_2O more than the 8 molecules indicated. The multiplication of both sides of the equation by 10, and the introduction of 1 molecule H_2O more, brings the theoretical expression in close accord with the facts, thus:

The following constitutional formula, which is similar in principle to that suggested by H. E. Armstrong many years previously, gives a representation of Brown and Millar's views on the constitution of the molecule of soluble starch, $100 \text{C}_{12}\text{H}_{20}\text{O}_{10}$.

On hydrolysis the dextran residue, marked A, constituting one-fifth of the whole molecule is split off with the formation of the stable dextrin, $39\text{C}_6\text{H}_{10}\text{O}_5, \text{C}_6\text{H}_{12}\text{O}_6$, whilst the maltan residues of the ring, marked B, are attached at the oxygen linkings of the C_{12} -groups, the hydrogen ions of the reacting water molecules moving in one direction and the hydroxyl ions in the other, thus forming, by successive stages of hydrolysis, maltodextrins or reducing dextrins and finally maltose.

V. Syniewski,¹ whose work will also be referred to later on, believes the starch molecule to be made up of amylogen residues (*see* below). From certain ingenious geometrical considerations, he concludes that the molecule of potato-starch consists of four amylogen residues, its empirical formula being $\text{C}_{216}\text{H}_{360}\text{O}_{180}$. The other starches are assumed to contain a different

¹ *Liebig's Annalen*, 1902 **324**, p. 212.

number of amylogen residues. The structure of the potato-starch molecule is thus represented :

Soluble Starch.—O'Sullivan¹ prepared this substance by dissolving starch at 73° to 74° C. (163° to 165° F.), with the least possible quantity of cold-water extract of malt, which had been heated to the same temperature. The solution was boiled, filtered, and concentrated by evaporation, when, as it cooled, soluble starch fell out as a brilliant white precipitate. It was further purified by being dissolved in water and re-precipitated therefrom several times. When dried it seemed to suffer some slight modification; a small portion was rendered insoluble, and another small portion (1 to 5 per cent.) converted into maltose and dextrin. The purest preparation he was able to make had an optical rotatory power of $[\alpha]_D + 220^\circ$ and a K of 0.78. Probably the reducing power was due to an impurity. Musculus and Gruber had previously described a similar body to which they assigned a K of 6.

In 1886 C. J. Lintner² stated that a soluble modification of starch could be readily formed by allowing ungelatinised potato-starch to remain in contact with 7.5 per cent. hydrochloric acid for seven or eight days. Starch when thus treated, and freed from acid by washing, dissolves in hot water without forming a viscid paste. Brown and Morris³ carefully examined the product of this reaction, and fully confirmed Lintner's observations. They also found that the same result could be attained in a much shorter time by using stronger acid. When 12 per cent. hydrochloric acid was used, the action was completed in twenty-four hours. The change in property is unaccompanied by any structural modification; the starch granules, when examined under the microscope, show no alteration in form; their behaviour towards polarised light is precisely similar to that before treatment. They also found that this body was identical with the soluble starch of O'Sullivan just described.

By heating starch with water under a pressure of two or three atmospheres, Ost obtained a nearly clear solution of a body which had a rotatory power of $[\alpha]_D + 196 - 197^\circ$, but no reducing power; it gave with aqueous solution of iodine in excess, a greenish blue colour, without any tendency to violet. The body separated from its hot aqueous solution on cooling, or could be precipitated from such a solution by alcohol, as a white powder, which, when recently precipitated, dissolved readily in cold water, but, after being dried over sulphuric acid, would only dissolve in hot water, and then

¹ *Journ. Chem. Soc. Trans.*, 1879, **35**, 772.

² *Journ. f. prakt. Chem.*, **34**, 378.

³ *Journ. Chem. Soc.*, 1889, **55**, 450.

not completely. Treatment of starch under higher pressures always led to the formation of dextrin. Ost considers that the term soluble starch will have to be restricted to starch which has passed into solution without the size of its molecule having been altered; in fact, he regards it as the same substance which passes into solution when starch is triturated with sand and water.

Soluble starch can be prepared, according to Zulkowski, by heating starch with glycerol to 190°C . (374°F). It can also be prepared by submitting starch and water to a temperature of from 125° to 150°C . (257° to 302°F .) in a closed vessel.

In 1897 Wroblewski¹ described a method of preparing soluble starch by treating starch granules with small quantities of a 1 per cent solution of caustic soda. The product is said to give a pure blue coloration with iodine, and not to reduce Fehling's solution. Syniewski² prepared soluble starch by treating potato-starch suspended in water with sodium peroxide. The product gave numbers on analysis agreeing with the formula $3\text{C}_6\text{H}_{10}\text{O}_5 \cdot \text{H}_2\text{O}$, or a multiple of it. It is readily soluble in cold water, but solutions stronger than 12.5 per cent. cannot be obtained at ordinary temperatures; it appears to dissolve in hot water in all proportions. The specific rotatory power observed in solutions of concentration varying from 2.5 to 12.5 per cent. was $[\alpha]_{\text{D}20^{\circ}} 186.1^{\circ}$. Brown, Morris and Millar³ give $[\alpha]_{\text{D}202^{\circ}}$ (see below) as the rotatory power of soluble starch prepared by Lintner's method. This difference, as well as the fact that solutions of Lintner's soluble starch, even of concentration as low as 2 per cent., become opalescent when kept, indicate that Syniewski's product is distinct from Lintner's. Syniewski⁴ further states that the simplest product of the carbinol hydrolysis of starch, obtained by heating the latter with water under pressure, or by the action on it of caustic potash or of sodium peroxide, is amylogen, $\text{C}_{54}\text{H}_{96}\text{O}_{48}$. It gives a pure blue coloration with iodine, and does not reduce Fehling's solution.

Properties.—Soluble starch, when prepared by any of the above methods, is readily soluble in water of 60° to 70°C . (140° to 158°F). When a strong solution (10 per cent.) is cooled down, the soluble starch separates out of solution as a white pasty mass (the same also happens when alcohol is added to a solution of soluble starch), which, on washing with alcohol and subsequent drying, becomes very friable. By submitting a dilute solution of soluble starch to a low temperature, it is possible to obtain the substance in the form of sphaero-crystals (*Lintner*). Soluble starch after once being dried is almost completely insoluble in cold water.

Soluble starch and its solutions are coloured an intense deep blue by iodine. Its specific rotatory power is given as $[\alpha]_{\text{D}33.66} + 216^{\circ}$ by Brown and Morris, and as $[\alpha]_{\text{D}} + 200^{\circ}$ by Lintner. Brown, Morris, and Millar⁵ state that the specific rotatory power of soluble starch in 2.5 to 4.5 per cent. solutions is, at 15.5°C ., $[\alpha]_{\text{D}} + 202^{\circ}$. It has no reducing action on Fehling's solution.

¹ *Berichte d. Deutsch. chem. Gesells.* 1897, 30, 2108. ² *Ibid.* 1897, 30, 2415.

³ *Journ. Chem. Soc. Trans.*, 1897, 71, 114. ⁴ *Liebig's Annalen*, 1899, 309, 282.

⁵ *Ibid.* 1897, 71, 114.

When acted on by diastase in the cold, it is degraded in ten minutes to the No. 8 equation (see pp. 80, 156, 161). When, however, the action of the diastase is allowed to proceed for ten or twelve days in the cold, soluble starch is said to be entirely converted into maltose.¹

The molecular weight of soluble starch as determined by Brown and Morris² employing the Raoult method, was from 20,000 to 30,000. According to the same observers, and on the assumption that the stable dextrin of the No. 8 equation constitutes one-fifth of the molecule of soluble starch, its molecular weight is 32,400, and its formula $5(C_{12}H_{20}O_{10})_{20}$. Brown and Millar³ represent the starch molecule by a constitutional formula which is given on p. 80.

Amylodextrins.—Nägeli, in his work on the starches, published in 1874, describes two amylodextrins which he had obtained by allowing 12 per cent. hydrochloric acid to act on starch for from two to four months, at the end of which time the starch granules had lost their power of giving a blue colour with iodine.

Amylodextrin No. I. was obtained by decanting the supernatant acid from the altered starch cells, and freeing them from the last traces of acidity by repeated decantation with water and alcohol. The granules, after being dried between sheets of blotting paper, were dissolved in hot water, the solution filtered, and then frozen. The amylodextrin separated out as a crystalloid substance, which, when examined under the microscope, was found to consist of round discs, each of which was composed of a mass of needle-shaped forms, grouped radially round the centre of the disc. These are the so-called sphæro-crystals. When examined by polarised light, they exhibited the phenomenon of double refraction.

Amylodextrin II. was obtained from the acid liquid in which the starch had been immersed, while this still gave a blue coloration with iodine, by adding to it after filtration four or five times its volume of 93 per cent. alcohol. The white precipitate was collected on a filter and washed with alcohol, dissolved in hot water, and the solution frozen; the amylodextrin then separated out in discs exactly similar to those of Amylodextrin I. The only difference between the two was their behaviour with iodine; a crystal of this substance communicated a blue-violet colour to No. I. and a red-violet to No. II. It was thought that they were not individual bodies, but mixtures of two bodies, one of which is coloured violet by iodine, the other red, and Nägeli succeeded in separating two bodies which fulfilled these conditions. Amylodextrin I. apparently contains more of the modification which gives a violet colour, whilst in No. II. the red-colouring modification preponderates.

Brown and Morris⁴ examined this substance very carefully, but were unable to fractionate it with alcohol. They found, contrary to Nägeli's assertion, that it was slowly but sensibly diffusible. They concluded that it was a perfectly distinct and definite compound, having an optical activity of

¹ Lintner, *Journ. f. prakt. Chem.* 1887.

² *Journ. Chem. Soc.*, 1889, p. 465.

³ *Ibid.* 1899, p. 336.

⁴ *Ibid.* 1899, p. 455.

$[\alpha]_D + 204.6^\circ$ and a κ of 10.12, corresponding to a composition of maltose 16.6 per cent. and dextrin 83.4 per cent. They regarded it as a compound of six dextrin groups and one maltose group, which would require an optical activity of $[\alpha]_D + 206.11^\circ$ and a κ of 9.08. Experiments made with Raoult's freezing method also point strongly to its molecular weight being 2286, a number which corresponds closely with the composition indicated. When treated with fresh malt-extract at any temperature under 60°C . (140°F .), it was completely hydrolysed to maltose, thus showing that it did not, like soluble starch, contain a dextrin residue. This curious fact led Brown and Morris to make a series of experiments to ascertain when the dextrin residue disappeared. The results of these are shown in the following table, in which potato-starch immersed in an 11 per cent. solution of hydrochloric acid was tested from time to time by treatment with fresh malt-extract :

Time of Treatment with HCl.	Iodine Reaction.	Apparent Composition.	
		Maltose.	Dextrin.
		Per Cent.	Per Cent.
After 48 hours . .	Pure blue	81.41	18.59
After 12 days . .	Reddish-purple . . .	87.70	12.30
After 21 days . .	Light reddish-brown . .	85.77	14.23
After 33 days . .	Light reddish-brown . .	90.10	9.90
After 66 days . .	Light reddish-brown . .	92.05	7.95
After 100 days . .	Light reddish-brown . .	92.24	7.76
After $8\frac{1}{2}$ years . .	Light reddish-brown . .	97.00	3.00

This last preparation, when dissolved in water and re-precipitated by alcohol, gave pure amylopectin, which, on treatment with fresh malt-extract, yielded maltose only.

The solution of hydrochloric acid used in the experiment was found to contain glucose equal in weight to 40 per cent. of the starch originally taken.

Maltose.—This sugar is produced whenever starch is submitted to the action of certain enzymes, such as diastase, ptyalin (from saliva), or trypsin (from the pancreas), under proper conditions. It is also one of the primary products of the action of acids upon starch.

Maltose was discovered by De Saussure in 1819, and again described by Dubrunfaut in 1847. These facts seem to have been forgotten until O'Sullivan in 1872 re-discovered the sugar and brought it prominently into notice (see p. 138).

Characteristics.—Maltose crystallises from its aqueous solution in the form of fine needles, which consist of sharply pointed prisms containing one molecule of water of crystallisation, $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$, which can be driven off by exposing the crystals to a temperature of 100°C . (212°F .) in a vacuum, when anhydrous maltose remains. Anhydrous maltose can also be obtained in crystals by slowly evaporating a strong alcoholic solution of the sugar.

Maltose is readily soluble in water ; its solution has a sweet taste. It is only slightly soluble in alcohol, much more so in alcohol containing water ; the more dilute the alcohol the greater is its solubility.

Rotatory Power.—The specific rotatory power of maltose has been variously stated by different observers. O'Sullivan,¹ using as solution divisor 3.85, gives $[\alpha]_j + 150^\circ$, and later on, using the more correct divisor 3.95, $[\alpha]_j + 154^\circ$. Brown and Heron,² using 3.86 as solution divisor, give $[\alpha]_j + 150^\circ$. When the requisite calculation is made, it will be seen that these last two numbers are almost identical. The absolute specific rotatory power of maltose as given by Brown and Heron (*l.c.*), who found that the correct solution factor for a 5 per cent. solution was 3.9314, is $[\alpha]_j + 153.1^\circ$, which equals $[\alpha]_D + 137.4$. Tollens has since given the value as $[\alpha]_D + 137^\circ$. Brown, Morris, and Millar³ give $[\alpha]_D = 137.93^\circ$ as the absolute specific rotatory power of maltose according to their latest investigations. According to Meissl, the rotatory power of a solution of maltose is little influenced by concentration, but considerably by temperature. He gives the following formula for making corrections for concentration and temperature :

$$[\alpha]_D = 140.374 - 0.01837P - 0.095T$$

in which P equals the percentage of maltose, and T the temperature of the solution. Thus a 10 per cent. solution at a temperature of 20°C. (68°F.) would have an optical rotatory power of $[\alpha]_D + 138.3^\circ$. A freshly prepared solution of maltose shows a rotation of some 18° less than the normal ; this, however, rises to the normal after the solution has stood for twenty-four hours, or it may be brought about instantaneously by the addition of a small quantity of solution of ammonia.

Cupric Reducing Power.—Maltose has a much lower reducing action on Fehling's solution than has glucose, only about two-thirds of the quantity of cuprous oxide being yielded by maltose which an equal weight of glucose would yield. O'Sullivan, in 1876, using a 3.85 solution factor, gave K65 as its reducing power ; Brown and Morris⁴ consider K61 with the 3.86 solution factor to be more correct ; and O'Sullivan, in 1884,⁵ using the 3.95 factor, gives 62.5, which, when calculated out to the 3.86 factor, gives 61.07. According to Soxhlet, with an excess of undiluted Fehling's solution and an approximately 1 per cent. solution of maltose, 100 parts of maltose invariably yield 127.3 parts of cupric oxide, or 113 parts of copper. A series of tables for the estimation of maltose have been prepared by Kjeldahl.⁶ Maltose has no reducing action upon Barfoed's solution (see p. 90, foot-note).

Action of Hydrolysts on Maltose.—Diastase has no hydrolysing action upon maltose, consequently this sugar is one of the lowest stages to which starch can be degraded by the action of diastase. Under certain conditions by the action of diastase on starch paste, dextrose is formed, this having

¹ *Journ. Chem. Soc.*, 1876, p. 479.

² *Ibid.* 1879, p. 619.

³ *Ibid.* 1896, p. 243.

⁴ *Ibid.* 1879, p. 619.

⁵ *Ibid.* 1884.

⁶ *Med. Carlsb. Lab.*, 1895, p. 1 ; *Analyst*, 20, 227.

been observed by Ling and Davis and by Syniewski. Brown and Heron found that the secretion of the Peyerian glands of the small intestine contained an enzyme which readily converted maltose into glucose. Dilute mineral acids, assisted by heat, hydrolyse maltose to glucose thus :

a reaction which is frequently made use of in the estimation of maltose in mixtures. Maltose is much less easily hydrolysed by acids than is cane sugar. According to Meissl, it offers five times as much resistance to hydrolysis as cane sugar.

Action of Yeast.—Maltose is easily and completely fermentable by yeast, but it is previously converted into glucose by the enzyme maltase contained in certain yeasts. Indeed only those yeasts which contain maltase can ferment maltose.

Osazone.—When maltose is treated with phenylhydrazine acetate, phenyl-maltosazone, $\text{C}_{24}\text{H}_{32}\text{N}_4\text{O}_9$, is formed, which precipitates, as the solution cools, in the form of yellow crystalline needles, soluble in hot water but insoluble in cold. The osazone melts at $190\text{--}200^\circ\text{C}$. with decomposition.

When heated with nitric acid, maltose yields saccharic acid,

when acted upon by bromine it yields maltobionic acid :

Constitution.—Maltose has been shown by Emil Fischer to be a disaccharide formed by the union of two glucose residues. These are probably united in the following manner :

consequently the molecule has only one free CHO group.

Glucose, Grape Sugar, or Dextrose.—This is one of the sugars found in great abundance in the vegetable kingdom. Ripe sweet fruits, such as grapes, plums, figs, &c., contain, in addition to fructose (lævulose), and small quantities of cane sugar, large quantities of glucose. It is met with in various other parts of plants, as the stems of the cereals, and in the flowers of many plants from which bees derive honey, a substance consisting largely of glucose. It is found in small quantities in the seeds of the cereals, such as barley. Compounds of glucose with numerous and very different substances are frequently met with in the vegetable kingdom. These are called glucosides, which, under the hydrolysing action of dilute acids or enzymes readily split up into glucose and their other constituents.

Preparation.—Glucose may be prepared in several ways, as, for instance by the inversion of cane sugar, or by the hydrolysis of starch, cellulose, dextrin, &c.

To secure glucose in a pure state, the former of these methods is generally used. Soxhlet gives the following directions for obtaining crystals of pure glucose. A mixture, made with 12 litres of 90 per cent. alcohol and 480 c.c. of fuming hydrochloric acid, is heated to 45° C. (113° F.), and into this is stirred 2 kilogrammes of powdered cane sugar, in small portions at a time, care being taken that the heat of the mixture never exceeds 50° C. (122° F.). In two hours the cane sugar is completely inverted—that is, converted into a mixture of glucose and levulose :

When the solution has become cold, a small quantity of crystallised glucose is stirred in, in order to start crystallisation. The whole is allowed to remain for twenty-four hours, at the end of which time the crystals are removed, washed with 90 per cent. alcohol until free from all traces of hydrochloric acid, washed with absolute alcohol, and recrystallised from pure methylic alcohol. In this way crystalline crusts of anhydrous glucose are obtained. If these crusts are melted with 12 per cent. of water on the water-bath, the glucose crystallises out on cooling. The crystals, after removal, are then dried over sulphuric acid; in this way hydrated glucose which crystallises with one molecule of water is obtained, $\text{C}_6\text{H}_{12}\text{O}_6 + \text{H}_2\text{O}$.

On the manufacturing scale, glucose is almost invariably made by submitting starch to the hydrolysing action of dilute sulphuric or hydrochloric acid assisted by heat. The process is conducted sometimes in open vessels, at other times in closed vessels under pressure; in either case, the apparatus is provided with some means of heating its contents, such as a steam-coil, or a steam-pipe perforated with small holes. The quantity of acid used per cwt. of maize or other material varies with different manufacturers, but is, as a rule, from six to eight pounds, and this is mixed with from forty to fifty gallons of water. Half the quantity of the dilute sulphuric acid required for the conversion is introduced into the converting vessel, and steam turned on until its contents boil. The ground degermed maize or other amylaceous material, intimately mixed with the remaining half of the dilute acid, is now admitted in a small stream, and the admission so regulated that the liquid in the converter never ceases boiling, by which means an equal distribution of the raw material is ensured. The whole is boiled, and during the latter part of the conversion the solution is frequently tested, first with iodine, and, when this ceases to give a reaction, with alcohol. When the addition of two volumes of alcohol to one volume of the cooled solution no longer causes turbidity, the boiling is continued for a little longer, when this portion of the operation is terminated. The solution of glucose is then run into another vessel, and the free sulphuric acid it contains neutralised by

the addition of whiting mixed with water. This latter is added in small portions at a time to prevent the frothing, due to the escape of carbon dioxide gas, becoming excessive, which would cause a loss of liquid by overflowing. When, on being tested with litmus paper, the liquid shows only a faint acid reaction, it is allowed to remain quiescent until the calcium sulphate has subsided. The clear liquid is drawn off from the precipitate, and that portion which adheres to the latter removed by filtration. The solution is then evaporated by being passed over an apparatus constructed after the manner of an upright refrigerator with steam circulating inside its tubes, until a concentration of about 58 to 60 per cent. of sugar is reached. During this process some calcium sulphate separates out, which is removed partly by subsidence and partly by filtration, after this the sugar solution is filtered through animal charcoal to remove colour and the more or less unpleasant taste and odour which it possesses. The solution is again subjected to further evaporation in a vacuum pan, until it has a concentration of about 80 per cent., when it is run into shallow vessels, where, under constant stirring, it is allowed to cool. The cooled semi-solid mass is then introduced into conical moulds holding from 30 to 60 lbs., in which it is left to solidify, afterwards it is cut into irregular-sized lumps by a machine in construction very much like a hay-chopper.

Glucose obtained in this manner is a somewhat hard, opaque, sweet substance, varying in colour from pure white to light brown, according to the amount of care expended in decolorising it by animal charcoal.

G. H. Morris¹ gives the following typical analyses of solid brewing glucose:

Dextrose	57.16	58.75
Maltose	8.09	6.49
Ash	1.45	1.18
Albuminoids	0.97	0.83
Water	15.70	15.83
"Gallisin" ² dextrin, &c., by difference	16.63	16.92
	<u>100.00</u>	<u>100.00</u>

The Brewers' extract is not given; but in such samples as these it should amount to about 72 lbs. per 2 cwt.

Soxhlet has shown that by increasing the amount of liquid to 9 parts of $\frac{1}{2}$ per cent. sulphuric acid for each part of starch, and heating the mixture for half an hour under a pressure of one atmosphere, a much better yield of glucose can be obtained than by the process as usually carried out. In this manner from 95 to 96 per cent. of the theoretical amount of glucose can be obtained. If the solution be evaporated in a vacuum to such a degree of concentration that it has a specific gravity of 1370 to 1380 (water = 1000) at 90° C., or, better still, a gravity of 1400 to 1420, the syrup slowly cooled

¹ *Journ. Fed. Inst. Brewing*, 1898, 4, 173, 174.

² The substances included under the term "gallisin" are probably reversion products.

down to 30° to 35° C., and kept at this temperature until complete solidification has taken place, it is possible to obtain the glucose as the hydrate, $C_6H_{12}O_6 + H_2O$, in a solid transparent form and with a distinct crystalline structure.

Rolfe and Defren¹ describe the manufacture of glucose from maize starch in the United States. The starch collected on the runs, and containing about 50 per cent. of water, is mixed with water to a thick cream of about 20° Beaumé. Conversion is carried out in large copper boilers at a steam pressure of 30 lbs., hydrochloric acid being the converting agent, the amount used being about 0.0006 the weight of the starch. The time of conversion is usually 40 minutes and is controlled by the iodine and alcohol tests as above described. As soon as the conversion is complete the liquid is blown out into the "neutraliser," where the alkali, usually sodium carbonate, is added. The other operations are conducted in the manner already described.

The manufacture of glucose is an important industry in this country, and at the present time not only solid glucose but also the so-called liquid glucose, or dextrin-maltose, is made in very large quantities in Great Britain. This last-named product is used extensively in confectionery as well as in brewing. It is an incompletely converted starch syrup, and therefore contains a considerable amount of dextrin in addition to glucose and maltose. When judiciously used it constitutes a most valuable brewing material.

The following are some typical analyses of dextrin-maltose :

	I.	II.	III.
Glucose	24.84	24.80	23.10
Maltose	20.12	22.00	26.03
Dextrin	37.42	34.62	32.17
Albuminoids	0.18	0.19	0.22
Ash	0.39	0.66	0.48
Water	17.05	17.73	18.00
	<u>100.00</u>	<u>100.00</u>	<u>100.00</u>
Brewers' extract per 2 cwt. .	71.9	71.6	70.7 lbs.

Characteristics.—Glucose crystallises from concentrated aqueous solutions, or from methylic or ethylic alcohol, in the anhydrous state, $C_6H_{12}O_6$. According as the process has taken place quickly or slowly, the sugar crystallises in fine needles or well-formed prisms, which belong to the triclinic system. Glucose also combines with water to form the compound $C_6H_{12}O_6 + H_2O$; the combination takes place slowly in the cold, but quickly if the solution be boiled. This sugar is very soluble in water, easily soluble in dilute alcohol, but difficultly soluble in absolute alcohol. Solutions of glucose rotate the polarised ray to the right, and concentrated solutions have proportionally a higher optical rotating power than dilute ones. Tollens has given the two following formulæ, the one for hydrated, the other for

¹ *Journ. Fed. Inst. Brewing*, 1899, 5, 59.

anhydrous glucose, by which the true optical rotatory power of a solution of any degree of concentration may be found. For $C_6H_{12}O_6 + H_2O$ —

$$[\alpha]_D = 47.73 + 0.015534P + 0.0003883P^2$$

and for $C_6H_{12}O_6$ —

$$[\alpha]_D = 52.50 + 0.018796P + 0.00051683P^2$$

in which P denotes the parts by weight of glucose in 100 parts by weight of the solution.

Glucose readily combines with oxygen, and hence is able to reduce the oxides of several metals to the metallic form; whilst in the case of some other metals, such as copper, the higher oxide is reduced to the lower, that is CuO to Cu_2O . On this property the methods for determining glucose quantitatively are founded (see p. 53). Glucose reduces Barfoed's solution in the cold.¹

Anhydrous glucose melts without decomposition at a temperature of $146^\circ C.$ ($295^\circ F.$); at a higher temperature it suffers decomposition, simultaneously losing water, and glucosan, a body having the formula $C_6H_{10}O_5$, is formed; when submitted to a still higher temperature, the mass gradually darkens in colour, and a dark brown body, caramel, is obtained. Oxidised with nitric acid, glucose yields saccharic and oxalic acids.

Treated with phenylhydrazine acetate, glucose yields an osazone which crystallises in beautiful yellow needles, which begin to separate out of the solution even while it is on the water-bath. Glucosazone melts at 204° to $205^\circ C.$, and is much more insoluble in either hot or cold water, or in alcohol, than maltosazone.

Glucose readily and directly undergoes the vinous fermentation, yielding in practice 48.67 per cent. of alcohol.

Glucose is an aldehyde sugar (an aldose) belonging to the mannitol group. Its constitution is expressed by the following formula— $CH_2OH \cdot 4(CHOH) \cdot CHO$. When acted on by nascent hydrogen, it is converted into sorbitol; when subjected to limited oxidation with bromine, it yields gluconic acid, $CH_2OH \cdot 4(CHOH) \cdot COOH$.

Birotation.—Dubrunfaut pointed out many years ago that freshly prepared solutions of glucose have about double the rotatory power of those which have been prepared for some time, and he isolated two forms of this sugar, one having specific rotatory power $[\alpha]_D 106^\circ$ and the other $[\alpha]_D 52^\circ$. Tanret² distinguishes these as α - and β -glucose respectively, and he has obtained a third modification, γ -glucose, the rotatory power of which is only $[\alpha]_D 22.5^\circ$. That the phenomenon of birotation (or, more correctly speaking, multirotation) was connected with these different modifications, which are interconvertible, was evident. It has long been known that when solutions of glucose are kept several hours, the specific rotatory power falls to a constant

¹ Barfoed's solution is prepared by dissolving one part of neutral crystallised cupric acetate in fifteen parts of water; to every 200 c.c. of this solution 5 c.c. of acetic acid, containing 38 per cent. of anhydrous acetic acid, are added.

² *Comptes rend.* 1895, 120, 1060.

amount which for analytical purposes may be regarded as the normal rotatory power; the fall in rotation is brought about immediately if the solution be boiled or if a minute trace of ammonia or alkali be added to it. These modifications of glucose represent cases of what H. E. Armstrong has called dynamic isomerism, and on this hypothesis it has been suggested by Lowry¹ that Tanret's β -glucose is merely a mixture of the α - and γ -modifications. What has hitherto been regarded therefore as the normal rotatory power of glucose is in reality produced by the attainment of a state of equilibrium between the α - and γ -forms, which, as already observed, are interconvertible. E. F. Armstrong, as well as T. M. Lowry, have recently suggested that in order to bring it into line with the analogous cases of isomerism presented by the so-called α - and β -glucosides, what has hitherto been called γ -glucose be now denoted β -glucose. Tanret accepts these suggestions, and taking the stable rotatory power of glucose in 10 per cent. solution as $[\alpha]_D^{52.5^\circ}$, he points out that this would represent 36.8 per cent. of the α - and 63.2 per cent. of the β -modification (formerly γ -modification) in equilibrium. The same explanation applies to other cases of multi- and mini-rotation. The validity of these theories receives its strongest support from the work of E. F. Armstrong² who has proved the analogy between the α - and β -glucosides and the α - and β -glucoses. This author, as well as T. M. Lowry, formulates the dynamic isomerides as γ oxides.

Lowry³ suggests that the interconversion of α - and β -glucose takes place through the medium of the aldehydrol (enolic form) which is formed in small quantity on dissolving glucose in water. The equilibrium may therefore be represented by the scheme :

Fructose, Lævulose, or Fruit Sugar.—This sugar, associated with glucose in invert sugar, is abundantly met with in nature.

Preparation.—Fructose can be prepared from inulin, a body which seems to take the place of starch as a reserve material in the tubers of certain plants, such as the dahlia, Jerusalem artichoke, &c. This body, on being hydrolysed with a dilute acid, yields fructose, just as starch under similar circumstances yields glucose. It has been shown that there is no series of intermediate bodies between inulin and fructose as there is between starch and glucose. As shown by A. Wohl,⁴ inulin, like cane sugar, can be inverted with very minute traces of hydrochloric acid (0.01 per cent. of the weight of ash-free inulin).

¹ *Journ. Chem. Soc. Trans.*, 1899, 212.

² *Ibid.* 1903, 1305.

³ *Ibid.* 1903, 1314; 1904, 1565.

⁴ *Ber. d. Deutsch. chem. Ges.*, 23, 2107.

For the preparation of crystalline fructose, Wohl directs 200 grammes of inulin to be introduced into a 500 c.c. flask with 50 c.c. of water. Hydrochloric acid is then added, in the case of a sample of inulin which contains 0.2 of ash, in the proportion of about one-half the percentage of the ash; when the ash amounts to from 0.2 to 0.4, then in a proportion of two-fifths of that percentage. The whole is heated in a boiling water-bath for half an hour, reckoned from the time the mass begins to soften; the free acid is then neutralised either by calcium carbonate or sodium carbonate, and the resulting syrup poured into a litre of warm (commercial) absolute alcohol, a little blood charcoal being added to remove colour, and the whole allowed to stand for twelve hours, at the end of which time the clear solution is decanted off from a small quantity of syrup which forms. The clear liquid, after filtration, is evaporated at a gentle heat under reduced pressure to a thick syrup, and this is mixed with three or four times its bulk of absolute alcohol, allowed to stand for twelve hours, and the solution decanted. A crystal of pure fructose is then introduced into the clear solution to start crystallisation, which is further promoted by stirring. At the end of twenty-four hours, pure anhydrous fructose, equal in weight to one-third of the syrup, is deposited. After three days a second crop forms, and by concentrating the solution by evaporation further quantities may be obtained.

Fructose may be also obtained from invert sugar by taking advantage of the fact that it forms a comparatively insoluble compound with calcium hydroxide, whilst glucose forms a soluble one.

Properties.—Fructose, crystallised from an alcoholic solution, forms a mass of fine brilliant silky crystals, which sometimes reach a length of 10 millimetres. Their melting point is 95° C. Fructose rotates the polarised ray strongly to the left, hence its original name, *lævulose*; but as it has been found that sugars of a similar constitution exist, which are either dextro-rotatory or inactive, it has been proposed by Emil Fischer to discontinue the name "*lævulose*," and use "*fructose*" instead.¹ The optical rotatory power of fructose is in a high degree dependent on the temperature of its solution; for a temperature of 20° C. with a 10 per cent. solution, *Parcus* and *Tollens*² give $[\alpha]_D - 92^\circ$ to $- 92.5^\circ$. Its reducing power is somewhat less than that of glucose. Fructose is completely fermentable by yeast, but does not ferment so rapidly as glucose. Treated with phenylhydrazine acetate it forms glucosazone identical with that obtained from glucose. Fructose is a ketone sugar (ketose), and its constitution may be expressed by the formula, $\text{CH}_2\text{OH}\cdot 3(\text{CHOH})\cdot \text{CO}\cdot \text{CH}_2\text{OH}$. When acted on by nascent hydrogen it yields mannitol and sorbitol. It is very easily decomposed, mere boiling with

¹ According to *Wiley (Journ. Amer. Chem. Soc., 1896, p. 81)*, the specific rotatory power of fructose at 0° C. [32° F.] is $[\alpha]_D - 108^\circ$, whilst at 88° C. (190° F.) it is equal to that of glucose, the alteration in the rotatory power being strictly proportional to the degree of temperature. This property furnishes a valuable method for estimating fructose in a mixture of other sugars.

² *Ber. d. Deutsch. chem. Ges., 24, 2000.*

water effecting its decomposition, as is evidenced, by its solution turning brown.

Cane Sugar or Sucrose.—This, the oldest and best known of the sugars, was originally derived from the sugar-cane (*Saccharum officinarum*), a native of the East Indies, the juice of which contains from 16 to 18 per cent. of sugar. Much sugar is now obtained from the root of the beet (*Beta vulgaris*), the juice of which contains 10 to 12 per cent. of sugar, and, by careful cultivation, this may be increased to 16 per cent. and upwards. It also occurs in the sap of many other plants, such as the sugar millet (10 to 11 per cent.), the stem of the maize plant (7 to 8 per cent.), and is probably one of the first products of assimilation in many plants. Cane sugar is found along with invert sugar in the juice of fruits and in many other parts of plants; also, as shown by Kuhnemann, and later by C. O'Sullivan, it is one of the sugars of barley and malt.

Properties.—Cane sugar crystallises in colourless anhydrous crystals, which belong to the monoclinic system, and which have a specific gravity of 1.58. It is very soluble in water; a saturated solution at 15° C. (60° F.) contains 66.3 per cent. of sugar, whilst a similar solution at 100° C. (212° F.) contains 82.5 per cent. It is very slightly soluble in absolute alcohol, more so in dilute alcohol, the solubility increasing with the dilution of the alcohol. It is insoluble in ether. The optical rotatory power of cane sugar in a 10 per cent. solution is $[\alpha]_D + 66.51^\circ$; it is higher than this in solutions more dilute, lower in solutions more concentrated. For solutions between 18 and 69 per cent. the following formula may be used: $[\alpha]_D = + 66.386^\circ + 0.015035P - 0.0003986P$, in which P equals the percentage of sugar in solution. It has no reducing action on Fehling's solution. Cane sugar melts at 160° C. (320° F.), and, on cooling, solidifies to an amorphous mass, which soon again becomes crystalline. When heated for some time to 170° C. (338° F.) it is split up into glucose and fructose (lævulose). When exposed to a higher temperature, 180° to 200° C. (356° to 392° F.), the mass becomes first yellow, then brown; in this latter condition it is known as caramel, a substance much used for colouring purposes. If heated to a still higher temperature, decomposition takes place, with evolution of combustible gases and acid vapours, a light porous mass of carbon being left behind (sugar-charcoal).

When cane sugar is exposed to the action of dilute mineral acids, it suffers inversion, and is converted into the mixture of equal molecules of glucose and fructose known as invert sugar. This process is much accelerated by heat. Invertase, an enzyme found in yeast, has likewise the power of inverting cane sugar.

Cane sugar forms compounds (saccharates) with several bases such as potassium, strontium, lead, &c., all of which are decomposed by carbonic acid. It does not form a compound with phenylhydrazine, but when heated in a solution of the acetate of that base, it first suffers hydrolysis into glucose and fructose, and both these bodies interact with the phenylhydrazine to form glucosazone.

Cane sugar is completely fermentable by yeast, but the fermentation is not a direct one, the sugar being first hydrolysed into glucose and fructose by the invertase of the yeast. Many bacteria, when introduced into cane sugar solutions, are able to induce peculiar fermentations, in which such bodies as lactic acid, butyric acid, mannitol, &c., are produced.

Difference between Beet and Sugar-Cane Sugar.—Sucrose derived from the sugar-cane is, especially after inversion, very largely used in the brewery as a partial substitute for malt. There are two sources of commercial cane sugar, the sugar-cane and the beetroot; the sugar derived from sugar-cane is generally used for brewing purposes, because cane sugar, so employed, is almost invariably used in its raw state. The impurities of the raw sugar derived from beetroot are of a nauseous character, whilst those of sugar-cane sugar have an agreeable, full, and luscious flavour; and so much is this the case, that the impure cane sugars are more valuable for brewing purposes than the refined, since the raw varieties yield to beer their luscious flavour. There is no objection, however, to the employment of refined beet-sugar¹ in the brewery.

Invert Sugar.—This sugar, which is a mixture of glucose and fructose, is found very abundantly in nature; it forms the chief constituent of honey; it is also contained in the juice of the grape and in many ripe fruits. It is produced by the hydrolysis of cane sugar by means of an acid or an enzyme, thus:

Preparation.—For this purpose the mineral acids are the most efficient, hydrochloric acid being the most powerful. As pointed out by Wohl,² a minute trace of this latter acid is, under certain conditions, able to effect the complete inversion of cane sugar. Thus, when to an 80 per cent. solution of the sugar 0.004 per cent. ($\frac{1}{2500}$ of a per cent. on the sugar) of hydrochloric acid is added, and the whole kept at the temperature of boiling water for an hour, with constant stirring, complete inversion takes place, and a pure colourless solution of invert sugar is obtained.

Carbonic acid is able to affect the inversion of cane sugar, for if a solution of this sugar be saturated with carbonic acid, inversion slowly takes place; but if the action is assisted by heat and pressure, complete inversion results in from twenty to thirty minutes.

Properties.—Invert sugar is met with as a thick syrup, from which, after a time, crystals of glucose separate. As invert sugar contains half its weight of fructose, which, at ordinary temperatures, is more powerfully lævo-rotatory than an equal quantity of glucose is dextro-rotatory, it also is lævo-rotatory. The optical rotatory power of a solution of invert sugar is much affected by

A patent has been taken out by Dr. Wohl, of Berlin, for removing the objectionable constituents of raw beetroot sugar by passing a current of air and steam through this substance when in solution.

² *Ber. d. Deutsch. chem. Ges.*, 1890, 23, 2084.

heat; when such a solution is gradually heated this gradually diminishes, until, when a temperature of 87.2°C . is reached, it is 0° ; if the solution be heated still further, it becomes right-handed. Tuschmid has given the following formula for ascertaining the specific rotatory power of a solution of invert sugar of any temperature :

$$[\alpha]_D - (27.9^{\circ} - 0.32t)$$

Its optical rotatory power is also influenced by the concentration of the solution, and for the correction of this O. Gubbe has given the following formula :

$$[\alpha]_D - 23.305^{\circ} + 0.01612.q + 0.00022391.q$$

in which q denotes the amount of water in the solution, therefore $100 - q$ denotes the amount of invert sugar.

The glucose constituent of freshly made invert sugar, when the latter is prepared by invertase, exhibits the phenomenon of birotation.

The reducing action of invert sugar on Fehling's solution is not so great as that of glucose (see p. 55).

Heated with phenylhydrazine acetate it yields glucosazone.

Invert sugar (when pure) is completely and directly fermentable by yeast. The glucose, however, ferments more rapidly than the fructose, as may be seen from the following table :

Time.	Glucose.	Fructose.	Difference in Milligrammes.
	Milligrammes per 100 c.c. Fermented.	Milligrammes per 100 c.c. Fermented.	—
After 20 hours	557	279	278
After 40 hours	968	532	436
After 64 hours	1196	721	475
After 86 hours	1392	942	450
After 108 hours	1616	1194	422
After 130 hours	1796	1423	373
After 158 hours	1970	1767	203

Invert sugar is by far the most important malt adjunct used in brewing and its manufacture constitutes a large industry in this country.

The following typical analyses of commercial invert sugar are given by Morris.¹

	I.	II.
Dextrose	37.39	37.48
Lævulose	29.53	31.81
Cane Sugar	0.80	1.18
Ash	1.59	1.54
Albuminoids	0.59	0.25
Water	22.21	21.38
Unfermentable matter, &c.	7.89	6.36
	<u>100.00</u>	<u>100.00</u>

This difference in the fermentability of the two sugars has been attributed by Bourquelot to the difference in their diffusive powers.

¹ *Journ. Fed. Inst. Brewing*, 1898, 4, 173.

Raffinose.—This sugar ($C_{18}H_{32}O_{16} + 5H_2O$) is found in a very small quantity in beetroots, and also, as shown by O'Sullivan, in barley. It crystallises in small needles or prisms, is easily soluble in water, slightly so in alcohol, and only possesses a faint sweet taste. Its optical rotatory power is $[\alpha]_D + 104.5^\circ$, and, like cane sugar, it has no reducing action on Fehling's solution. When heated for a short time with a dilute acid, it is hydrolysed into equal molecules of fructose and of a dissaccharide isomeric with milk-sugar called "melibiose." This latter sugar, on prolonged treatment with a dilute acid, is by further hydrolysis converted into equal molecules of galactose and glucose.¹ These reactions show that raffinose is a trisaccharide, that is, its molecule contains three C_6 groups; the glucose and galactose residues being united in a similar manner to that in which they combine to form milk-sugar, whilst the fructose residue is probably attached in some similar manner to that in which it is united with glucose in cane sugar. On fermentation raffinose behaves differently with the various yeasts; the ordinary top fermentation brewery yeasts are able to hydrolyse and ferment it completely, since they contain both the enzymes invertase and melibiase, whilst bottom fermentation yeasts, which generally only contain invertase, partially invert it to melibiose and fructose, the latter sugar being alone fermented.

α - and β -Amylan.—These bodies were isolated by O'Sullivan from barley, wheat, and rye. Barley contains about 2 per cent. of α -amyln and about 0.3 per cent. of β -amyln. They were obtained by extracting barley, first with alcohol, then with water. The aqueous solution was concentrated by evaporation, and strong alcohol added, which precipitated the two bodies. The precipitate was then treated with cold water; this dissolved out the β -amyln and left the α -amyln. The latter was afterwards dissolved in dilute hydrochloric acid, and precipitated therefrom by alcohol. Both are lævo-rotatory, and have the following optical rotatory powers:

α -Amylan	$[\alpha] - 24^\circ$
β -Amylan	$[\alpha] - 73^\circ$ (increasing to -146°)

On being hydrolysed with dilute acids they are said to be converted into glucose. It is probable that these substances are mixtures of pentosans and hexosans, and that their hydrolytic products consist to some extent of pentoses. At the time of their isolation pentosans as such were not known.

Gum.—Lintner² has isolated from beer, and also from barley and malt, a gum very similar to that which can be extracted from wood by a dilute solution of caustic alkali, and which is named "xylan." It is also found in straw, bran, and brewers' grains. The gum was isolated from beer by treatment with dilute caustic alkali and cupric sulphate, the latter combining with the gum to form an insoluble precipitate. When obtained in the pure state,

¹ Scheibler and Mittelmeier. *Ber. d. Deutsch. chem. Ges.*, **22**, 1678 and 3118.

² *Brauer und Malzer Kalendar*, 1890-91, ii. p. 121.

the gum forms a loose white powder, which is slowly soluble in cold water but quickly soluble in hot. It does not, however, form a true solution, but the particles are merely finely suspended in the water. Even dilute solutions are very viscous, and filter with great difficulty. The gum is dextro-rotatory, does not reduce Fehling's solution, and is precipitated by lead acetate. Like many other gums, it gives a cherry-red colour with phloroglucinol and hydrochloric acid, also a bluish-green colour with orcinol and hydrochloric acid; its presence in beer may be demonstrated by these reagents. As shown by Lintner and Düll,¹ it may be regarded as a galactoxylan; for when boiled with a dilute acid it is resolved into galactose and xylose. Evidently it is formed by the union of a molecule of galactose with a molecule of the pentose, xylose, with the elimination of a molecule of water, thus :

L. Lindet² isolated two gums from germinating barley. One having a specific rotatory power of $[\alpha]_D - 146^\circ$, which he regards as identical with the β -amylan of C. O'Sullivan; it gave a mixture of pentoses on hydrolysis; and a second having a rotatory power of $[\alpha]_D + 84.6^\circ$, and a cupric reducing power of 30-35, which appeared to be identical with the α -galactan isolated by Muntz from lucerne seeds. The amount of the latter is said to increase during germination, whilst the amount of the supposed β -amylan remains constant.

Furfural (*Furfur*, *Bran*).—This is an oily liquid of an aldehydic nature,

which boils at 160°C. and has the constitution $\begin{array}{c} \text{HC—HC} \\ || \quad || \\ \text{HC} \quad \text{HC—CHO} \\ \diagdown \quad \diagup \\ \text{O} \end{array}$, from which

the corresponding alcohol and acid may be prepared. It is best obtained by distilling a mixture of bran with an equal weight of sulphuric acid diluted with three volumes of water. Furfural gives a beautiful red colour with aniline acetate, and when treated with phenylhydrazine acetate yields furfural phenylhydrazone. One of the peculiar properties of the pentoses is that when boiled with a dilute acid they yield furfural. Consequently, when galactoxylan is boiled with dilute hydrochloric acid, furfural is invariably formed. It is also formed to a slight extent during the process of mashing. The free acids of the mash first hydrolyse the galactoxylan, and other carbohydrates containing a pentose complex, and then act on the pentoses produced to form furfural. Furfural can be detected in beer and in distilled spirits, notably in whisky and brandy.

Pectin.—This is a substance nearly allied to the gums; it occurs in ripe juicy fruits, such as apples, pears, &c., also in roots, such as turnips, carrots, &c. If the juice of any of these substances be boiled to remove the coagulable albuminoids, filtered, and alcohol and hydrochloric acid added, a jelly-like precipitate of pectin falls. The formula of this body is $\text{C}_{32}\text{H}_{45}\text{O}_{32}$, as given by

¹ *Zeit. angew. Chemie*, 1891, p. 538.

² *Comptes rend. de l'Acad. des Sciences*, 1903, 137, 73.

Fremy, who looks upon it as a transformation product of another body contained in unripe fruits named "pectose." An enzyme, pectase, is said to effect this transformation. Ullick has isolated substances of this nature from beer by rendering it strongly alkaline with caustic soda solution. A precipitate forms, which may be separated into two portions by treatment with dilute hydrochloric acid. The portion soluble in hydrochloric acid is precipitated by the addition of a large quantity of absolute alcohol to the acid solution, the precipitate washed with alcohol, and purified by being dissolved several times in water and reprecipitated therefrom by alcohol. In this way a white body is obtained, soluble in cold water, and having an optical rotatory power of $[\alpha]_D + 113^\circ$. The portion difficultly soluble in hydrochloric acid is washed with cold water and dissolved in boiling water. A strongly coloured solution is obtained in this way, which, on being decolourised with sulphurous acid, yields a precipitate, which, when again dissolved in water has an optical rotatory power of $[\alpha]_D + 288^\circ$. Another pectinous body with a rotatory power of $[\alpha]_D + 36^\circ$ still remains in the sulphurous acid solutions. Similar substances have been found in apples, sugar-beetroots, and ordinary beetroots. These bodies possess in a high degree the property of yielding viscous solutions, and, as the palate-fulness of a beer is dependent up to a certain point on viscosity, the pectinous bodies must contribute in some degree to its palate-fulness.

The Fatty Constituents of Barley.—Many seeds, such as linseed, rape-seed, castor-oil seeds, &c., contain as a reserve material large quantities of oil, and in no seed is oil or fat entirely absent. Barley contains about 2.5 per cent., rye about 2.0 per cent., whilst linseed contains 37 per cent., and rape-seed 45 per cent. Most fats are glyceryl esters of the fatty acids. Thus stearic acid reacts with glycerol to form stearin; palmitic acid in the same way forms palmitin; and oleic acid, olein. When any one of these bodies is subjected to the action of a caustic alkali, a salt of the fatty acid and glycerol results, thus:

Such a process is called "saponification," and on it is based the manufacture of soap.

The fat of barley can be readily obtained from the ground corn by extraction with ether. On removal of the ether by evaporation, a yellowish-brown limpid oil is left behind, which possesses an agreeable odour; but this is soon lost and replaced by an offensive one if the oil is allowed to remain in contact with the air. On standing, the barley-oil separates into two nearly equal portions; one of these is liquid at the ordinary temperature, whilst the other solidifies to a mass of crystals. The composition of barley-fat has been investigated by a number of observers. König and Kiesel found it to contain olein, stearin, and palmitin, and also oleic, stearic, and palmitic acids. Lintner, senior, has shown that it contains cholesterol, a monatomic alcohol of the composition $\text{O}_{26}\text{H}_{43}(\text{OH})$, which is a normal constituent of animal bile. This is

an unsaponifiable body, in no way allied to the ordinary fats. It is fatty in appearance, insoluble in water, tasteless and odourless. It dissolves readily in boiling alcohol, in ether or chloroform, and is deposited from boiling alcohol, on cooling, in the form of beautiful white scales, which have a lustre like mother-of-pearl. Cholesterol melts at 137°C. , and sublimes at 200°C. A. Stellwag has detected the presence in barley-oil of lecithin, a body containing phosphorus, and which has the following composition, $\text{C}_{42}\text{H}_{84}\text{NPO}_9$. According to this observer, the composition of barley-oil is as follows:

	Per Cent.
Free fatty acids	13.62
Neutral fat	77.78
Lecithin	4.24
Cholesterin	6.08

F. Beckmann found that on distilling brewers' grains with dilute sulphuric acid a volatile fatty acid passes over with the steam, which is found as a white substance floating on the water in the receiver. He named it "hordeic acid." It is insoluble in water, but readily dissolves in alcohol or ether; it crystallises from this latter solvent in irregular scales, which are unaltered on exposure to the air.

THE PROTEÏDS OR ALBUMINOIDS.

A large number of substances occurring in the animal and vegetable kingdom, which are intimately associated with living matter, are included under the term "Proteïds," or, as they are often called, the "Albuminoids." These bodies contain the elements nitrogen and sulphur, in addition to carbon, hydrogen, and oxygen. The different proteïds have often been submitted to elementary analysis, but the results obtained by different observers, when analysing what was presumably the same substance, have not, until recently, been very concordant. This arose from the difficulty which existed of obtaining the different members of the proteïd group in a state of purity; it is only within comparatively recent times that this has been accomplished with any degree of success.

Reactions of the Proteïds.—These bodies give the following characteristic reactions, which may be readily obtained with a solution of egg-albumin made by mixing a small quantity of white of egg, which contains about 12 per cent. of albumin, with water and filtering the solution.

Precipitation by Nitric Acid.—When strong nitric acid is added to the aqueous solution of any of the proteïds, a white precipitate forms, which turns yellow on heating the liquid. The addition of ammonia to the mixture after it has become cold causes the yellow precipitate to become orange-coloured.

Biuret Reaction.—On adding a few drops of a very dilute solution of cupric sulphate to the aqueous solution of a proteïd, and afterwards a few drops of a

strong solution of caustic soda, a colour is developed which varies with the different classes of proteïd. The albumins give a violet colour; the albumoses or proteoses, a reddish-violet; the peptones, a rose-red.

Millon's Reagent.—This, which is a solution of mercuric nitrate in nitric acid, yields, with proteïds, a white precipitate which becomes reddish on boiling the fluid.

Evolution of Ammonia.—The proteïds when strongly heated evolve ammonia, produced under the destructive action of heat. The ammonia may be recognised by its changing the colour of a piece of moist litmus paper from red to blue. A smell resembling that of burnt hair is also given off during the heating process.

Formation of Cyanides.—When heated with metallic sodium, the proteïds yield sodium cyanide. The presence of this compound can be detected by extracting the mass with water, adding a few drops of a solution of ferrous sulphate containing a little ferric sulphate, and digesting for a short time. On the addition of hydrochloric acid a blue or bluish-green colour is produced if proteïds are present.

Molecular Constitution of the Proteïds.—As to the nature of the molecular constitution of the proteïds we are still in the dark. Undoubtedly the proteïd molecule is a very large one, and, although some insight has been obtained as to the nature of the different molecular groups which combine to form the large complex molecule of the proteïd, yet we know comparatively little as to how these groups are actually combined with one another. In the determination of the size of the proteïd molecule, as the cryoscopic method is inapplicable, advantage has been taken of certain metallic compounds which the proteïds form. For instance, in the compound of egg-albumin with copper it has been found that $C_{204}H_{322}N_{52}O_{66}S_2$ combine with one atom of copper. From the magnesium, sodium, and calcium compounds, which have been obtained with the crystalloid globulins obtained from various seeds, the formula $C_{292}H_{481}N_{90}O_{83}S_2$ has been deduced. Whether these values are true or only approximative, they serve to show that the proteïd molecule is a very large one.

Effects of Hydrolysis on the Proteïds.—When proteïds are submitted to the action of certain hydrolysing agents, such as superheated steam, dilute mineral acids, caustic alkalis, or the enzyme trypsin, the large proteïd molecule becomes finally split up into much smaller and less complex molecules; and it is from the study of these fragments, as it were, that we have obtained some little knowledge as to what is the nature of the molecular groups which enter into the composition of the proteïds. By the action of boiling hydrochloric acid, to which a little stannous chloride has been added, which combination possesses an extremely powerful hydrolysing action, the proteïd molecule can be broken down into comparatively small decomposition products, such as leucine, tyrosine, aspartic acid, glutamic acid, gluco-protein, lysine, and lysatine. When barium hydroxide is used as the hydrolysing agent, in addition to the formation of the before-mentioned products, much

ammonia and carbon dioxide are evolved; and these gases are given off in the proportion of one molecule of water and one of urea, $\text{CO} \begin{matrix} \text{NH}_2 \\ \text{NH}_2 \end{matrix} + \text{H}_2\text{O} = \text{CO}_2 + 2\text{NH}_3$.

When proteïds are acted upon by certain enzymes, the degradation process does not extend so far as this. The molecules of the bodies produced are much nearer in size to that of the original proteïd molecule. Of such a nature are the albumoses or proteoses and peptones.

Synthesis of Proteïds.—Although the synthesis of a true proteïd has not actually been accomplished, the recent classical researches of Emil Fischer and his colleagues have taught us a great deal as to the structure of these complex substances. Among the reactions characteristic of the proteïds is the so-called biuret reaction referred to above. In 1883 T. Curtius¹ prepared a substance from glycocoll ester, which gave the biuret reaction; it has been termed the “biuret base,” and it has since been shown to be the ethylic ester of a polypeptide:

Polypeptides have been prepared synthetically by Emil Fischer and his colleagues from residues of glycocoll or glycine, leucine, tyrosine, &c. Fischer commenced this important work by synthesising and separating the various amino-, and oxamino-derivatives of the acids of the fatty series. The di-, tri-, and poly-peptides are obtained by linking together residues of these acids. These compounds have many reactions in common with the natural peptones. Some of them have been found to be of a racemoid nature and have been resolved into dextro- and lævo-isomerides; others have been found to be susceptible of attack by proteolytic enzymes, whilst they all give the biuret reaction. Typical of these peptides are the following compounds:

The Various Members of the Proteïd Groups.—It has been found possible to isolate the proteïds from the substances with which they are associated, and to effect a fairly complete separation of the various members of the proteïd groups from one another, by taking advantage of the varying solvent powers which different saline solutions exercise upon them, and also of the property possessed by certain salts of throwing out of solution certain members of the group when the solution is saturated with the particular salt. This latter process is technically known as “salting out,” and by its employment we are able to divide the various plant proteïds into the following groups:

¹ *Ber. d. Deutsch. chem. Ges.*, 1883, 16, 755.

Albumins.—Soluble in water, coagulated by heat.

Globulins. (*a*) **Vitellins.**—Insoluble in pure water, soluble in dilute saline solutions, coagulable in greater part by heat. (*b*) **Myosins.**—Insoluble in pure water, soluble in dilute saline solutions, precipitated on saturation of the solution with sodium chloride, coagulable by heat.

Gliadin and Hordein.—Slightly soluble in water, readily soluble in 70 per cent. alcohol.

Glutenin.—Slightly soluble in hot water and hot alcohol, soluble in 0.1 per cent. solution of caustic potash, and in 0.2 per cent. hydrochloric acid, insoluble in saline solutions.

All these bodies, with the exception of the globulins, are obtained in an amorphous condition, in which state they probably exist in the plant. Many of the globulins have been obtained in a crystalloid form, and to some extent at least they exist in this condition in some seeds. Crystalloid proteïds can be readily observed in the Brazil-nut and in the seed of the castor-oil plant. Cube-shaped proteïd crystalloids occur in the potato, and may readily be seen by placing under the microscope a portion of the layer which is situated immediately below the skin of a boiled potato. If the starch cells are treated with iodine, the proteïd crystals, since they are only stained yellow, can be readily distinguished from the blue starch grains.

The Proteoses.—These bodies are derived from the proteïds by hydrolysis, and are also characterised by their behaviour when their aqueous solutions are saturated with certain salts. Thus a sharp distinction can be drawn in this way between the proteoses and the peptones, for if an aqueous solution of these two bodies is saturated with ammonium sulphate, the former are precipitated, the latter remains in solution. By taking advantage of similar properties, the group of the proteoses, as will be seen further on (page 105), can be separated into three members—proto-proteose, deuteroproteose, heteroproteose, &c.

The Proteïds of Barley.—Hitherto these bodies have received but scant attention on the part of chemists, the proteïds of wheat having much more frequently formed the subject of chemical investigation. Mulder¹ found that barley contained 6 per cent. of albumin and plant gelatin; he obtained the latter by extracting barley-meal with hot alcohol. Von Bibra² considered that the proteïd constituents of barley were albumin, plant gelatin, and casein, but gave no particulars concerning these substances. Kreusler found that the aqueous extract of ground barley contained an albumin which coagulated on boiling, and that hot 75 per cent. alcohol dissolved a substance which could be subsequently separated into three proteïds, gluten-casein, gluten-fibrin, and mucedin, which were supposed to be identical with the bodies having the same names that, with another body, gliadin, Ritthausen³ had isolated from wheat.

¹ *Physiol. Chem.*, i. 306.

² *Die Getreidearten*, &c., p. 204.

³ *Die Eiweisskörper*.

Isolation of the Proteïds of Barley.—An elaborate series of investigations on the proteïds of various grains and seeds have been made by T. B. Osborne,¹ and we are indebted to him for much valuable information as to the nature and properties of the proteïds occurring in these substances. The general methods of procedure employed will be briefly described, taking for this purpose his investigations into the proteïds of the barleycorn.

Separation of the Albumin or Leucosin.—A large quantity of barley, say six or seven pounds, is ground into fine meal and extracted with 10 per cent. solution of sodium chloride. This dissolves out the albumin, globulin, and the proteose. If an attempt is made to dissolve out the albumin with water and leave the globulin behind, it does not succeed, for there are always a certain amount of soluble salts present in barley or other cereals, which dissolve in the water employed, and yield a dilute saline solution capable of dissolving the globulin. The salt solution thus obtained is next saturated with ammonium sulphate; this precipitates all the proteïds in solution. These, after being filtered off, are again dissolved in 10 per cent. sodium chloride, the solution filtered and dialysed for several days, when the whole of the globulin is precipitated. Since this body is insoluble in pure water, it is precipitated when the salt has been removed from the solution by dialysis. The precipitated globulin is then collected on a filter, and the clear filtrate gradually heated to 65° C. (149° F.), which has the effect of coagulating and precipitating the albumin. The precipitated albumin is now filtered out, washed with warm water, alcohol, and ether, and dried over sulphuric acid.

Separation of the Globulin or Edestin.—The separation of the globulin is somewhat difficult, since the salt solution extracts a large amount of gum along with it; and this difficulty is increased by the readiness with which globulin passes into an insoluble condition. It was prepared in as pure a state as possible by dissolving the globulin, obtained in the above-mentioned manner, in a 10 per cent. salt solution, and again dialysing; the operations of solution and dialysation being repeated a second time. The proteïd then separated in the form of small spheroids, which were filtered off, washed with water, alcohol, and ether, and dried over sulphuric acid. The purified substance, when dissolved in a 10 per cent. salt solution and gradually heated, became turbid at 90° C. (194° F.), but no coagulum actually formed until the solution actually boiled, and even then only a small portion of the substance was precipitated.

Separation of the Hordeïn.—In the first experiments on the isolation of this substance barley-meal was employed, but as this was found to yield a large quantity of colouring matter, ground pearl-barley—that is, barley from which the husk has been removed—was employed. From a quantity of this the bodies removable by a 10 per cent. solution were first extracted, and then sufficient alcohol added to the ground pearl-barley, while still wet with salt solution, to form with the water retained by the meal alcohol of 75 per cent.

¹ Report of the Connecticut Agricultural Experiment Station, 1894.

After this extract had been removed from the meal, another extraction was made with 75 per cent. alcohol. The two extracts were united, filtered, and concentrated to about a third of their bulk by evaporation on the water-bath. The proteïd, which separated out as a plastic mass, was, after decanting off the supernatant fluid, macerated with distilled water, this poured off, and the hordeïn dissolved in 75 per cent. alcohol. The solution of the proteïd thus obtained was poured into a quantity of distilled water, when the substance in solution separated. After removal of the water, the precipitated proteïd was again dissolved in 75 per cent. alcohol, and the solution poured into a large quantity of absolute alcohol. The proteïd did not separate, even after the addition of ether in considerable quantity, owing to the entire absence of mineral salts, these having been completely removed by the previous treatment. The addition of a little sodium chloride solution caused its immediate precipitation, the alcohol and ether retaining any fat which had been extracted along with the proteïd. The precipitated hordeïn was then treated with successive portions of absolute alcohol and dried over sulphuric acid. Obtained in this way, it is a snow-white granular substance, which, when completely free from alcohol, is almost insoluble in cold water, and slightly soluble in hot. Hot aqueous solutions do not yield a precipitate on cooling, nor do they coagulate on boiling, though they yield precipitates, not inconsiderable in amount, on the addition of salt. When hordeïn is dissolved in strong hydrochloric acid, it gives a beautiful crimson solution. When dissolved in a mixture of water and sulphuric acid in equal volumes, its solution has a red colour; a similar solution of wheat gliadin has a purple-red colour.

Proteïd Insoluble in Water, in Saline Solution, and in Alcohol.—The quantity of this substance contained in barley-meal was ascertained by estimating the amount of nitrogen the meal contained after having been extracted with salt solution and alcohol; this quantity was then multiplied by the factor 5.9 on the assumption that the proteïd substance contained 17 per cent. of nitrogen. Attempts to dissolve out the proteïd with a weak solution of caustic potash resulted in failure. The previous treatment to which the barley-flour had been subjected apparently had rendered it almost insoluble, and the small amount which did dissolve was so much contaminated with gum that its further purification was impossible. Barley contains these various proteïd bodies in about the following proportions:

	Per Cent.
Leucosin (albumin)	0.30
Proteose	1.95
Edestin (globulin)	
Hordeïn	4.00
Insoluble proteïd	4.50
Total	10.75

Average Percentage Composition of the Proteïds of Barley.—These are the means of a large number of analyses:

—	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.
Leucosin	52.81	6.78	16.62	1.47	22.32
Edestin	50.88	6.65	18.10	24.37	
Hordein	54.29	6.80	17.21	0.83	20.87
Insoluble proteid . .	Unknown.				

Gluten Group.—It has long been known that, on kneading the dough of wheat-flour under a stream of water, a tough, coherent, elastic mass is left behind, which can be pulled out into strings. This is the gluten, or the body which gives the coherent character to the dough made from wheat-flour, and the presence of which confers on that flour the property of yielding a light porous bread. Gluten cannot be obtained in this way from the meal of any other grain. It has been the subject of much investigation. Einhof¹ in 1805 discovered that a certain portion of the proteid matter of wheat could be extracted with hot alcohol, and this he considered to be identical with gluten. Various other observers worked in the same direction, notably Ritthausen,² who was able by fractionation to differentiate the substances extracted from wheat-flour with alcohol into three bodies—gluten-fibrin, gliadin, and mucedin, and a fourth, gluten-casein, which was insoluble in alcohol, but soluble in dilute alkaline solutions. He considered that these four bodies constituted the gluten of wheat, and that it was the gliadin which formed the binding material. This body was found to be absent in the flour of those grains which left no gluten behind on washing.

Weyl and Bischoff³ have been led to the opinion that these bodies did not exist as such in the seed, but that the proteids of the wheat and other grains consisted chiefly of a globulin substance, which, when the flour was moistened for the purpose of extraction, was converted into the gluten bodies through the agency of an enzyme. Osborne and Vorhees,⁴ in an exhaustive examination of the proteids of wheat, have come to the conclusion that this is not the case, but that the gluten bodies exist ready formed, though their number is not so large as had been hitherto supposed, there being but two, gliadin and glutenin. Ritthausen (*loc. cit.*) considered that there were three bodies belonging to the gluten group in barley, viz., gluten-fibrin, gluten-casein, and mucedin. Osborne, as we have previously mentioned, finds that there are only two: hordein, which is apparently identical with the substance called "mucedin" by Ritthausen, but which has almost the same physical and chemical properties as the gliadin obtained from wheat, though it differs from it in composition; the second is the insoluble proteid which it was found impossible to isolate.

¹ *Journ. d. Chemie von Gehlen*, 5, 131.

² *Die Eiweisskörper*.

³ *Ber. d. Deutsch. chem. Ges.*, 13, 367.

⁴ *American Chemical Journal*, 15, 392.

The Proteïds of Maize.—The maize corn was subjected to an exhaustive examination by Chittenden and Osborne in 1891 and 1892, who found that it contained three globulins, one or more albumins, and a proteïd soluble in alcohol. Direct extraction with water dissolved out a myosin-like globulin, but owing to the presence of soluble salts in the maize, the solvent was in reality a dilute saline solution. Further extraction with a 10 per cent. salt solution extracted a vitellin-like globulin. The third globulin was characterised by its extreme solubility in very dilute solutions of various salts, and especially of phosphates and sulphates. The first of these globulins, when dissolved in a 10 per cent. sodium chloride solution, coagulated at about 60° C. (140° F.); the second, when dissolved in a similar solution, hardly coagulated at all under the action of heat, but was precipitated on the addition of acetic acid; the third globulin coagulated in a 10 per cent. salt solution at about 62° C. (144° F.). By prolonged contact with water these globulins were gradually converted into insoluble modifications, apparently albuminates. Water also extracts from maize two albumin-like bodies, the first of which, when its solution is gradually heated, separates at between 60° and 70° C. (140° and 158° F.); a portion of the second separates when the liquid boils, but the whole of this proteïd could not be removed in this way, probably because it became transformed into albumose. The only representative of the gluten group found in maize was a body termed "maize-fibrin" or "zeïn." This substance is extremely soluble in 75 per cent. alcohol, but is insoluble in either water, saline solutions, or dilute alkaline solutions.

The Proteïds of Malt.—The proteïds of barley undergo considerable modification during the process of malting; a large portion which are insoluble in water become soluble after the barley has germinated. This arises chiefly from the breaking down of the proteïds into proteoses by the action of the proteolytic enzymes.

The proteïds of malt have been submitted to an exhaustive investigation by Osborne and Campbell.¹ By employing processes similar to those which they had used in the examination of the proteïd matters of barley, they obtained the following bodies:

Bynedestin.—A globulin, soluble in very dilute saline solutions, and therefore passing into the aqueous extract of malt, which is in reality a weak solution of the salts contained in the malt. It appears to replace the original edestin of the barley, from which it differs somewhat in composition, since bynedestin contains about 2 per cent. more carbon and 3 per cent. less nitrogen. It has the following percentage composition: Carbon, 53.19; hydrogen, 6.69; nitrogen, 15.68; sulphur, 1.25; oxygen, 23.19.

Leucosin.—Identical in composition and properties with the albumin of the same name contained in barley.

Protoproteose I. has the same composition as leucosin, from which it is impossible to effect a complete separation. The proteose is precipitated from its aqueous solution by adding an equal weight of alcohol.

¹ Report of the Connecticut Experimental Station, 1896, p. 239.

Protoproteose II.—Less readily precipitable than No. I. by alcohol. It has the following composition: Carbon, 50.63; hydrogen, 6.67; nitrogen, 16.69; sulphur and oxygen, 26.01. It differs, therefore, considerably in composition from No. I.

Deuteroproteose.—A body which could not be separated from non-proteid impurities.

Heteroproteose.—A substance found in extremely small amount.

Bynin.—Insoluble in water or saline solutions, but readily soluble in dilute alcohol. It has the following composition: Carbon, 55.03; hydrogen, 6.67; nitrogen, 16.26; sulphur, 0.84; oxygen, 21.20.

Insoluble Proteid.—This, which amounted to 3.80 per cent. of the total proteid matters, was insoluble in water, saline solutions, or alcohol; consequently it was impossible to study its composition or to determine its properties.

A sample of malt which contained altogether 7.84 per cent. of proteid matters yielded the following quantities of these substances, so far as they could be separated:

	Per Cent.
Proteid, insoluble in salt solution or in alcohol	3.80
Bynin, soluble in dilute alcohol	1.25
Bynedeſtin, leucosin, and proteoses } Coagulable	1.50
soluble in water and salt solution } Uncoagulable	1.29
Total proteids	7.84

These results show “that in germination the proteids of barley undergo extensive changes before acquiring the properties of proteoses; that hordein disappears, and an alcohol-soluble body of entirely different composition takes its place; that edestin also disappears, and a new globulin is formed very different both in composition and properties. The albumin, on the other hand, appears to be unchanged in its character, but its quantity is increased. It is to be noted also that hordein and edestin are both replaced by proteids much richer in carbon and poorer in nitrogen.”

Coagulation Temperatures of the Proteids of Malt.—Solutions of bynedeſtin (globulin) become turbid when heated to 65° C. (149° F.); flocks begin to form when the temperature has risen to 84° C. (183° F.); and these gradually increase in amount as the temperature rises to the boiling-point. The whole of the globulin is not, however, completely thrown out of solution even at this temperature, since the addition of dilute hydrochloric acid still produces an abundant precipitate.

The albumin is completely precipitated at a temperature of 59° C. (136° F.); consequently, this substance will be coagulated and left in the mash-tun. The bynin and insoluble proteid, being both insoluble in water or weak saline solutions, are of no moment to the brewer, though it is probable that, during their stay in the mash-tun, they may be converted to some slight extent into proteoses by the proteolytic enzymes of the malt.

At present we are unable to make use of the indications afforded by the

relative proportion of the nitrogenous matter of malt contained in these groups, because so far no observations have been carried out to show how malts, in which these relative proportions differ from the normal, behave in the production of beer. But it is fair to presume that if this subject were thoroughly investigated in the direction indicated, important criteria would be established for the valuation of samples of malt.

According to the experiments of Hantke,¹ the following percentages of the total amount of the nitrogenous matter of a malt which he examined passed into solution at the following temperatures :

	Per Cent.
16 hours' mash at 72° F.	31.78
1 hour's mash at 100° F.	52.16
1 hour's mash at 122° F.	37.51
1 hour's mash at 145° F.	32.75
1 hour's mash at 167° F.	32.80

Consequently the largest quantity is extracted at 100° F.

Of the total nitrogenous matter taken into solution by mashing at the following temperatures, the following percentages were precipitated on boiling the wort for an hour :

	Per Cent.
16 hours' mash at 72° F.	21.12
1 hour's mash at 100° F.	15.24
1 hour's mash at 122° F.	14.18
1 hour's mash at 145° F.	22.80

From this it appears that at a mashing temperature of 122° the smallest amount of nitrogenous matter coagulable on boiling is extracted ; apparently, therefore, this temperature is the most favourable one for peptonisation.

THE AMIDE CONSTITUENTS OF PLANTS.

In addition to the proteïds all vegetables contain another set of nitrogenous bodies, the amides, which, being of a crystalline nature, are eminently diffusible. We have already mentioned some of these bodies as being amongst the products of the digestion or breaking down of the proteïds, but it is extremely probable that the amides in the living plant form a portion of the material from which the proteïds are constructed. The amides themselves are probably produced by a combination of the deoxidisation products of the chlorophyll cells with nitrogen, derived from the nitrates and ammonia contained in the liquid contents of the cells.

Asparagine.—One of the most frequently occurring of these amide bodies, and the one which has been longest known, is asparagine, so called because it was first found in asparagus sprouts. It forms large colourless crystals, readily soluble in water, but insoluble in alcohol. It has the following composition : $C_4H_8N_2O_3 + H_2O$. An aqueous solution of the ordinary asparagine

¹ *Brewer and Maltster*, 1895, p. 1148.

rotates the polarised ray to the left, its optical rotatory power being $[\alpha]_D - 6.23^\circ$ but a dextro-rotatory asparagine which rotates to the same angle in the opposite direction has been found in tares by Piutti. The dextro-rotatory body has a distinctly sweet taste; the lævo-rotatory is almost tasteless. When boiled with dilute acids asparagine is readily transformed into aspartic acid and ammonia, thus: $C_4H_8N_2O_3 + H_2O = C_4H_7NO_4 + NH_3$. The same reaction ensues when it is boiled with a solution of a caustic alkali; only in this case the aspartic acid itself becomes hydrolysed to malic acid and ammonia, and even in simple aqueous solution asparagine suffers slight hydrolysis on prolonged boiling. Asparagine, when acted on by nitrous acid, suffers decomposition, and yields half its nitrogen in the gaseous state, according to the following equation:

Sachsse¹ has based a method for the estimation of asparagine, and the amides generally, on this reaction. Asparagine is precipitated by mercuric nitrate, and the compound so obtained may be decomposed into asparagine and mercuric sulphide by the action of hydrogen sulphide.

Aspartic Acid.—This is a crystalline body, slightly soluble in water, which rotates the polarised ray to the right or left, according as the asparagine from which it was obtained was dextro- or lævo-rotatory. Treated with nitrous acid, aspartic acid yields up the whole of its nitrogen, thus:

Glutamine.—This is a crystalline body, having the following composition, $C_5H_{10}N_2O_3$. It readily suffers decomposition, being readily split up by the action of alkalis or acids, or by prolonged boiling of its aqueous solution, into glutaric acid and ammonia. In consequence of its unstable nature, glutamine is seldom met with in plants, though the frequent presence of glutaric acid leads to the presumption that this latter body is a decomposition product of glutamine. When treated with nitrous acid, glutamine is decomposed in accordance with the following equation:

Glutaric Acid is a crystalline substance, slightly soluble in water, its solution being dextro-rotatory. A lævo-rotatory acid is also known. The former acid is found amongst the products of the hydrolysis of the proteïds.

Tyrosine.—This body, which is hydroxyphenylamidopropionic acid, $C_6H_4 \begin{smallmatrix} \text{OH} \\ \text{OH} \end{smallmatrix} \text{CH}(\text{NH}_2)\text{COOH}$, crystallises in fine brilliant silky needles, soluble with difficulty in cold water, slightly more so in hot water, but insoluble in alcohol. Its aqueous solution rotates the polarised ray to the left; a dextro-rotatory

¹ *Agricult. Chemie*, p. 390.

modification is also known. Treated with nitrous acid, it yields up nearly the whole of its nitrogen. It has long been known to be one of the products of proteolysis, and is frequently met with in germinating seeds.

Leucine or Amido-Caproic Acid.—An amide present in the products of proteolysis, and which is also found in germinating seeds. It consists of brilliant silvery-white plates, soluble in about twenty-seven parts of cold water, and slightly soluble in alcohol. Its aqueous solution is dextro-rotatory. It has been prepared synthetically. When treated with nitrous acid, it yields up the whole of its nitrogen, thus :

Vernine.—An amide occurring in plants and having the composition $\text{C}_{16}\text{H}_{20}\text{N}_5\text{O}_8 + 3\text{H}_2\text{O}$. It has been isolated by Schulze and Bosshard. It crystallises in brilliant silky needles, dissolves with difficulty in cold water, readily in hot, but is insoluble in alcohol. Vernine, when heated with hydrochloric acid, yields guanine $\text{C}_5\text{H}_5\text{N}_5\text{O}$.

Betaine.—Another body of an amide nature, but which perhaps ought rather to be classed with the alkaloids. It has been isolated by Schulze from beetroots. Its formula is :

Choline.—An alkaloidal substance which possesses a bitter taste, is of an exceedingly hygroscopic nature, and has an alkaline reaction. Its composition is $\text{HO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{N}(\text{CH}_3)_3\cdot\text{OH}$. It readily combines with carbon dioxide, and can be formed artificially from trimethylamine and glycol. It has been found in hops, in wort, and in beer.

The Amides Occurring in Malt.—Ullik was able to detect hypoxanthine, guanine, and vernine in wort and beer, also probably leucine and tyrosine, but he was unable to find either asparagine or glutamine. Amthor found vernine and another substance which appears to have a composition intermediate between xanthine and guanine. Düll¹ found the amine body choline in barley and malt. Asparagine appears to be contained in malt rootlets, but not in the malt itself.

THE ENZYMES OR HYDROLYSTS.

Enzymes.—There are found in all living organisms, be they animal or vegetable, certain remarkable nitrogenous bodies which are either actually albuminoids or are very closely allied to them. They are soluble in water and are coagulated by heat. Each one possesses the power of splitting up the complicated compound molecule of some particular organic body, such as starch,

¹ *Chem. Zeit.*, 1893, p. 67.

albumin, &c., when the enzyme and the substance are brought into intimate contact with each other under certain conditions, an absolutely necessary one of these being the presence of water. These nitrogenous bodies are often spoken of as the "unorganised ferments" or "enzymes," and the transformations they effect termed "fermentative processes." When, in the changes which are brought about through their agency, water is added to the molecule of one or both of the newly-formed substances, the process has been called "hydrolysis," and the agents concerned in the action "hydrolysts."

The selection of the name "ferment" for these bodies, and that of "fermentation" for the processes they induce, was an unhappy one, since these terms had been associated with an entirely different set of bodies and processes, *i.e.* what were considered to be organised ferments, such as yeast, bacteria, &c. So much is this the case, that when fermentation is spoken of, one is extremely apt to connect this term with the older process denoted by that name, and hence confusion arises. When the enzyme of an organism, such as yeast, acts upon sugar in solution, the appearances of boiling are more or less present; and since the word "fermentation" is derived from the Latin word *fervere* (to boil), it would seem much the more reasonable course to reserve this word for the designation of those processes where gas is evolved and with which it has been associated the longest, this being more in accordance with its original signification. Those enzymes which bring about the hydrolysis of substances may be termed "hydrolysts";¹ and by combining with the word "hydrolyst" the name of the substance on which the particular enzyme acts, we obtain a distinctive name for each class. Thus the enzymes which act upon starch are called "amyl-hydrolysts"; those that act on proteids, "proteo-hydrolysts," &c. (Compare, however, footnote on p. 112.)

Action of Enzymes Similar to that of Acids.—Actions similar to those caused by enzymes can be induced by several other agents. Sulphuric, hydrochloric, oxalic, and many other acids, apparently act upon starch in a precisely analogous manner to that in which the amyl-hydrolyst diastase acts. Quite recently H. Neilson² has shown that platinum black hydrolyses starch. During the process the acid neither suffers loss nor decomposition, and can be entirely recovered at the end of the experiment in an absolutely unaltered condition. Several metallic oxides, such as those of copper, cobalt, &c., behave in a similar manner with certain compounds which contain oxygen, such as hydrogen peroxide and the hypochlorites. They decompose these bodies when in solution, with the liberation of oxygen, the metallic oxides themselves suffering no change whatever in the process.

Law of Mass Action.—The hydrolysis of starch, cane-sugar and other polysaccharides by dilute acids has been shown to proceed in such a manner

¹ Suggested by Professor Armstrong (*Journal of the Chemical Society*, 1890, p. 528).

² *Amer. Journ. Physiol.*, 1906, 15, 412.

that the amount of sugar hydrolysed in a given interval of time bears a constant proportion to that remaining unchanged. This is represented by the following formula $\frac{1}{\theta} \log \frac{1}{1-x} = k$, in which k = the constant proportion of polysaccharide above referred to, θ = the time and x = the amount of polysaccharide hydrolysed in θ . In other words, the course of the change may be expressed by a logarithmic curve. Wilhelmy in 1850 demonstrated that the hydrolysis of cane-sugar by dilute acids follows this law. Quite recently E. F. Armstrong and R. J. Caldwell have shown, however, that when very dilute acids (*e.g.* $\frac{N}{500}$ hydrochloric acid) are employed, the amount of cane-sugar hydrolysed is in direct proportion to the time, so that the change may then be represented by a straight line. When the course of the reaction proceeds according to the ordinary law of mass action it is said to be logarithmic, whilst when the amount of change is in direct proportion to the time it is said to be linear.

The Rate of Change Conditioned by Sucroclastic Enzymes.¹—

C. O'Sullivan and F. W. Thompson² published experimental evidence showing that the hydrolysis of cane-sugar by the invertase of yeast follows the law of mass action. Duclaux, however,³ contended that the rate of change during the early period is linear, although under the influence of the products of hydrolysis it subsequently follows the logarithmic law. V. Henri⁴ also attributes the departure from the logarithmic law to the influence of the products of hydrolysis. A. J. Brown⁵ shows that the velocity of the action is linear when not influenced by the accumulation of the products of hydrolysis. He agrees with Henri⁶ that the value $2k$ is approximately constant when derived from the expression $2k = \frac{1}{\theta} \log \frac{1+x}{1-x}$. In any simple chemical change the influence of mass regulates the number of molecular contacts between acting and reacting molecules in unit time; but, assuming that during the hydrolysis the sugar enters into molecular combination with invertase, this presupposes a complex change and the existence of a time factor of some magnitude which would limit the influence of mass. But if this be so there must be a point of dilution when the hydrolysis by invertase should be in conformity with simple mass action, and Brown shows that this point is reached in a solution containing about 1 per cent. of cane-sugar.

¹ Professor H. E. Armstrong has pointed out that the terms "amylolytic, proteolytic," &c., are confusing to the student who has learnt that electrolysis signifies splitting up by means of electricity, and hydrolysis by means of water—not splitting up of electricity or of water. He suggests that enzymes should be spoken of as "sucroclastic, glucosidoclastic, amylolastic, proteoclastic, lipoclastic," &c., according as they hydrolyse disaccharides, glucosides, starch, proteids, fat, &c.

² *Journ. Chem. Soc., Trans.*, 1890, 57, 834. ³ *Annales de l'Inst. Pasteur*, 1898.

⁴ *Lois générales de l'Action des Diastases*, Paris, 1903.

⁵ *Journ. Chem. Soc., Trans.*, 1902, 373.

⁶ *Loc. cit.*

From the classical researches of Kjeldahl in 1897,¹ it follows that the action of malt diastase on starch is in the first stages a linear function of the time, for it is shown that the hydrolysing powers of two malts are proportional when both act on the same quantity of starch at the same temperature, provided the reaction has not been allowed to proceed beyond the stage at which the copper-reducing power indicates the production of more than 45 per cent. of apparent maltose. Assuming that malt diastase is invariable in its action it may therefore be deduced from this law of Kjeldahl's that the first stage of the hydrolysis of starch by diastase is a linear action. Kjeldahl it may be observed used kilned malts in his experiments, and Ling has pointed out that the law does not hold for green malts. H. T. Brown and Glendinning² confirm Kjeldahl's law. They show that if the time curves expressing the rate of hydrolysis of a 3 per cent. solution of soluble starch are critically examined, it is found that up to a hydrolysis of 30 to 40 per cent. the amount of transformation is very nearly a linear function of the time, whilst the remainder of the reaction is approximately logarithmic. It is very difficult to utilise the data (optical rotatory and cupric-reducing powers) obtained from the later stages of starch transformations by diastase on account of the fact that the nature and relative amounts of the products are unknown; besides this there is some evidence that a change in the reverse direction occurs after a certain time.

E. F. Armstrong has studied the action of lactase and of emulsin on milk-sugar, and of yeast-maltase on maltose.³ His results, taken in conjunction with those of A. J. Brown and of H. T. Brown and Glendinning (see above), support the view that the rate of change is essentially dependent on the relative proportions of enzyme and sugar. Assuming that the enzyme combines with the sugar, the effect of varying the conditions may be predicted and is in harmony with observed facts; thus, if the amount of enzyme is small relatively to the initial amount of sugar, the change is at first a linear function of the time; as the hydrolysis proceeds and the amount of enzymes is relatively greater, the change is approximately a logarithmic function of the time as required by the law of mass action. If the amount of enzyme is initially considerable compared with the amount of sugar, the linear part of the change is not apparent. When the amount of enzyme decreases during hydrolysis, owing to the influence of the products, the rate of change will be governed by an equation of the second order, so that the velocity constant k calculated for the simple logarithmic formula will gradually diminish. If the amount of enzyme and water are kept constant, an increase in the amount of sugar beyond that required for complete combination with the enzyme will not augment the quantity of hydrolyte undergoing change in a given time. The proportion of hydrolyte to the value of k will, however, decrease as the sugar concentration is increased.

¹ *Comptes rend. des trav. du lab. de Carlsberg*, 1897, i. 109.

² *Journ. Chem. Soc., Trans.*, 1902, 388.

³ *Proc. Royal Soc.*, 1904, 73, 500 and 516.

Armstrong finds that the hydrolysis of milk-sugar by emulsin is considerably retarded by dextrose, slightly by galactose, and not perceptibly by levulose. The relative effect of dextrose, galactose and levulose on the hydrolysis of maltose by maltase is the same as that just mentioned. Hydrolysis by invertase, on the other hand, is retarded by levulose, but not by dextrose. According to this author these results show the closely-related configurations of enzyme and of hydrolyte, and may be taken as evidence that the enzyme combines with the hexose in such a way that it is withdrawn from the sphere of action.

Armstrong also points out that Emil Fischer's researches have proved that the naturally occurring hexoses or derivatives of these, *i.e.* glucose, mannose, galactose, and fructose, are the only compounds affected either by the organisms which condition alcoholic fermentation or by any of the sucroclastic enzymes. The stereoisomeric hexoses produced by artificial means cannot be fermented; this is true also of the "lower" and "higher" sugars, whether derived from natural products (arabinose, xylose, &c.), or prepared artificially from the natural products by reducing or adding to the number of carbon atoms in the chain. The fact that resistant material such as straw, the gums, &c., are pentose derivatives is of interest in this latter connection. Three of the four hexose sugars referred to are fermented readily and apparently with equal ease; the fourth, galactose, is only slowly fermented; such being the case, it is noteworthy that the formulæ ordinarily assigned to glucose, mannose and fructose are reducible to one common enolic form, and it may be that this enolic form is the substance actually fermented to carbon dioxide and alcohol.

Ordinary dextrose in solution consists almost wholly of two stereoisomeric compounds in equilibrium (see p. 91) together with a small proportion of the enolic form.

Both T. M. Lowry and E. F. Armstrong have brought forward strong arguments based on experimental results that a γ -oxide formula must be assigned to glucose and its derivatives. In the formula now to be given when R=H it is that of glucose itself, whereas when it is an alkyl radical it is one of the alkyl glucosides. When R is a hexose residue (C₆H₁₂O₆-H), it represents a biose, such as maltose. The carbon atom to which the group RO is attached in the formula is the superior carbon atom in the formula above given. The effect of hydrolysis whether by acids or enzymes is to remove the radical R and displace it by hydrogen. In the case of enzyme action it may

be assumed that the enzyme becomes attached in some way along the line of carbon atoms, thus—

Purposes for which Enzymes are Produced.—The important work in nature assigned to the hydrolytic enzymes is the conversion of insoluble bodies into soluble and diffusible ones, or to render diffusible those bodies of a non-diffusible nature which already exist in solution. If these necessary changes did not take place, many substances could not be made use of by animal or vegetable organisms. For instance, when germination commences in a grain of barley, it is necessary that the starch stored up in its endosperm should be able to travel to the growing germ. This is first effected by an amylo-hydrolyst (the translocation diastase) already present in the seed; afterwards by another hydrolyst ordinary diastase), which is formed in considerable quantity during germination. The insoluble proteid substances in the grain are also rendered soluble and diffusible in a similar manner by another set of enzymes, the proteo-hydrolysts. But enzyme action is not confined to the process of germination alone, for enzymes are to be found in every part of the living plant, leaves, buds, tubers, stem, &c.

One of the most striking facts connected with the enzymes is that an exceedingly minute quantity of any one of them is able to transform an enormous amount of the substance on which it is capable of acting. The mechanism of the hydrolysis has not yet received an absolutely certain explanation; the most probable conjecture is, that they are bodies whose molecules are in a high state of vibration, and that they are able to transmit these vibrations to other bodies in their immediate neighbourhood with such force as to overcome the stability of the molecules of the latter bodies. These are, as it were, shaken into bodies which possess a simpler and more stable molecular condition, upon which the vibrations of the enzyme are no longer capable of exercising a disruptive action.

Enzymes Derived from the Albuminoids.—If the hydrolysts are not themselves albuminoids, they are undoubtedly near derivatives of them, being most probably oxidation products. It is, however, somewhat doubtful whether they are really independent entities, or merely albuminoids which in a certain phase of their development temporarily assume this power. Amylo-hydrolysts are found in some organs of the plant which never contain starch, and where, in such cases, their presence is inexplicable unless they are made use of afterwards in the nutrition of the plant. It can hardly be imagined that the energy and material necessary for the production of these bodies would be expended for a totally useless purpose. The change from albuminoid to enzyme does not seem to be of a very profound nature. Baranetzky found that an aqueous extract of the resting tubers of the potato did not possess any

amylolytic power when freshly prepared, but that, after remaining for a few days exposed to the air, the solution acquired this property, which it, however, again lost on further keeping. That this alteration could not be attributed to the access of bacteria is shown by the fact that the solution remained tasteless and odourless throughout the experiment, and at the end of it possessed the same slight degree of acidity which it did at the commencement. According to Sachsse,¹ the simplest view to take of enzymes, if we do not regard them as albuminoids, is to look upon them as near derivatives of these bodies, easily formed from them by the action of oxygen, and which just as readily resume their original condition. Viewed in this light, they are not exclusively formed for the specific purpose of inducing hydrolytic changes, but are general and normal products of the albuminoids produced by respiration in the plant. That oxidation plays an important part in the formation of the hydrolysts in the seed is shown by the well-known fact that seeds, when caused to germinate in a thick layer to which the oxygen of the atmosphere can only obtain but limited access, produce much less diastase than do similar seeds when they are spread out in a thinner layer and more freely exposed to the air. The maximum quantity of the enzyme is formed in seeds sown under natural conditions in the earth, since in this case each seed is isolated and secures an abundant supply of oxygen.

It has been shown by Reychler that when bodies belonging to the gluten-casein group are subjected to the action of dilute acids, a body possessed of amylolytic properties is formed, to which he gives the name of "artificial diastase." Lintner and Eckhardt, who have also investigated this subject, corroborate the results of Reychler, and find that this is especially the case with mucedin. When four grammes of mucedin were dissolved in 100 c.c. of 0.1 per cent. acetic acid, and the solution immediately tested for diastatic power, this was found to be 35.7°. On standing at a temperature of 35° C. (95° F.), the amylolytic power of the solution gradually increased, until at the end of twenty-one days it had risen to 83.3°. A similar and equal effect was produced on the mucedin in ten days when dissolved in a 0.1 per cent. solution of hydrochloric acid, or in a 0.2 per cent. solution of acid potassium phosphate H_2KPO_4 . This effect, they suggest, may be caused by the action of the acid on an unknown "zymogen"² present in the seed.

Brown and Heron have shown that when an aqueous extract of barley is submitted to the action of yeast for a few hours at a temperature of 30° C. (86° F.) its power of transforming starch is much increased. This they attribute to some change brought about in the soluble albuminoids by the yeast.

In a state of complete dryness the enzymes will bear exposure to a

¹ *Agricult. Chemie*, p. 364.

² A zymogen is a body which under certain conditions may become converted into an enzyme.

temperature of 100° to 125° C. without losing the whole of their characteristic properties.

The Various Groups of the Enzymes.—Each particular hydrolytic enzyme can only act upon a certain class of substances; indeed it has been shown by Emil Fischer that there is a relation between the configuration of an enzyme to the substance it acts upon. Thus, diastase readily hydrolyses starch, but has no action on proteïds; similarly pepsin, which hydrolyses proteïds, is unable to affect starch. The enzymes may be divided into the following seven groups, in which they are arranged according to their respective specific actions :

GROUP I.....Diastatic Enzymes :

Diastase of secretion (malt)	}	Hydrolyses starch into maltose, glucose, and maltodextrins.
Translocation diastase (barley)		
Ptyalin (saliva)		
Glucose (maize)	{	Hydrolyses starch finally into glucose.

GROUP II.....Cyto-hydrolytic enzymes :

Cytase (malt)	}	Transform cellulose into sugars, such as mannose, xylose, &c.
Enzymes of seeds in which the reserve material is cellulose		

GROUP III...Pectin enzymes :

Enzymes which convert pectinous substances into vegetable jelly.

GROUP IV....Inverting enzymes :

Invertase (yeast)	}	Convert cane-sugar into invert sugar.
Invertase (malt)		
Maltase (yeast)	}	Transform maltose into glucose.
Enzyme of the small intestine		
Enzymes of yeast which degrade the malto-dextrins to maltose ; these are especially present in those yeasts which bring about the secondary fermentation and condition of beer.		
Enzyme of kephir		Inverts milk-sugar.
Probable enzyme of germinating barley	{	Converts maltose into cane-sugar.

GROUP V....Proteolytic enzymes :

Enzymes of malt and other vegetables (sometimes called peptase), but which have not yet been isolated	}	Convert proteïds into proteoses, peptones, and amides.
Trypsin (pancreas)		
Pepsin (stomach)	}	Converts proteïds into proteoses and peptones, but not into amides.
Peptonising ferments secreted by many bacteria.		

GROUP VI....Glucosidal enzymes :

Emulsin (bitter almond)	{	Splits up amygdalin into oil of bitter almonds, hydrocyanic acid, and water.
Many other enzymes which have the power of hydrolysing glucosides.		

GROUP VII...Zymase of yeast :

Splits up sugar into alcohol and carbon dioxide.

The Diastases or the Amylolytic Enzymes.—The substance of this nature, which has been known for the longest time, is the diastase found in germinated barley; it was also the first enzyme isolated, though it was only obtained in an exceedingly impure condition. Since then it has been discovered that enzymes of this class are widely disseminated throughout the vegetable kingdom; scarcely a plant, or part of a plant, has been examined without detecting their presence. As has been shown by Lintner and Eckhardt, and by Brown and Morris, two distinct forms of diastase exist; one of these is present in considerable quantity in ungerminated barley, the other, which is secreted during the germination of barley, is largely present in malt.

Isolation of Diastase.—Numerous attempts have been made from time to time to obtain malt diastase in a state of purity, the first investigations on record in this direction being those of Payen and Persoz. Their method was as follows: A strong cold-water¹ extract of malt was heated for half-an-hour to a temperature of 70° C. (158° F.), in order to coagulate certain albuminous matters; the extract so treated was then filtered off, and a large quantity of absolute alcohol added to the filtrate, which precipitated the diastase. The precipitate thus obtained was again dissolved in water, reprecipitated by alcohol, and dried at a temperature of 40° to 50° C. (104° to 122° F.). A white tasteless and odourless powder was obtained in this way, which, upon solution in water, was found to possess amylolytic properties. The diastase produced in this way is very weak in its action, since it suffers great deterioration at the high temperature to which the malt-extract is subjected. Various other attempts have been made from time to time to improve the methods of isolating diastase, such as that of extraction with glycerin, followed by precipitation by alcohol; precipitation by common salt, by acetate of lead, &c., but all these methods have proved unsatisfactory. A better process, which is in reality a modification of the method of C. O'Sullivan, published in 1884, was proposed by C. J. Lintner. Lintner directs one part of ground barley-malt or of sieved green malt to be digested for twenty-four hours or longer with two parts of 20 per cent. alcohol.² The solution is then filtered off, and twice, or at most two and a half times its volume of absolute alcohol added, with constant stirring. More than this quantity of alcohol should not be added, or matters, possessed of little diastatic activity, are simultaneously precipitated. The precipitated diastase quickly settles in yellowish-white flakes, from which the supernatant liquid is poured off, the precipitate brought on to a filter, and the alcoholic fluid removed as quickly as possible. The residue is removed from the filter-paper,

¹ By cold-water extract of malt is meant a solution of the soluble portions of malt in cold water. It is prepared by grinding malt to a fine powder in a mill and mixing it with cold water in a beaker or other vessel. The mixture, after standing for a certain length of time, during which it is occasionally stirred, is filtered off. Malt-extract contains in solution the diastase and other soluble nitrogenous constituents, gum, ready-formed sugars, and also a certain amount of maltose formed by the action of the diastase on the starch granules during the extraction.

² Syikágyi employs 30 per cent. alcohol, so as to dissolve less inert proteid matter.

placed in a mortar and well triturated with absolute alcohol, again placed on the filter, the alcohol removed and the precipitate washed with absolute alcohol. The precipitate is brought once more into the mortar, triturated with anhydrous ether, placed on the filter, and the ether removed as far as possible by the suction-pump, after which the precipitate is dried in a vacuum over sulphuric acid. The above-mentioned dehydration of the diastase by alcohol and ether is absolutely necessary in order to obtain the substance in the form of a loose powder, easy of solution, and possessing the maximum diastatic activity. If the dehydration is incomplete, the preparation dries to a tough horny mass, which is only partially soluble, and is possessed of little diastatic activity. By repeated solution in water and re-precipitation by alcohol the enzyme may be entirely freed from carbohydrate matters.¹ The mineral constituents, which in the freshly precipitated preparation amount to 16 per cent., are exceedingly difficult to remove, but can be reduced by dialysis to a little less than 5 per cent. The most powerful diastase which Lintner was able to prepare in this manner, and presumably the purest, had the following percentage composition: C46.66, H7.35, N10.41, S1.12, O34.46. It differed, therefore, considerably from the ordinary proteid in composition, being much richer in oxygen and poorer in nitrogen.

A most important series of investigations on diastase have recently been carried out by J. B. Osborne,² which have considerably advanced our knowledge of this substance. The diastase was prepared in the following manner: Ten kilogrammes of ground malt were extracted with water, and the malt-extract thus obtained saturated with ammonium sulphate. This had the effect of precipitating the whole of the proteids in solution. The proteids were then suspended in four litres of water, and dialysed until much of the ammonium sulphate had been removed. The dialysed solution, after being filtered off from a residue consisting chiefly of globulin which had become insoluble, was again saturated with ammonium sulphate. The precipitate so obtained was suspended in 1500 c.c. of water, submitted to dialysis, and filtered off. Most of the globulin was converted by this treatment into the insoluble modification and remained behind. The solution of the malt proteids, *minus* nearly the whole of the globulin, was now dialysed into alcohol of specific gravity 0.84. In forty-eight hours the precipitate which had formed was removed; it was called "Precipitate No. 1." The solution was again dialysed into alcohol of the same strength for another forty-eight hours, when another precipitate formed; this was removed and called "Precipitate No. 2." The filtrate from this was dialysed into a rather larger quantity of somewhat stronger alcohol, when "Precipitate No. 3" formed and was filtered off. The filtrate from this was similarly treated when "Precipitate No. 4" was obtained. To the filtrate from this a large quantity of alcohol was added,

¹ As shown by its solution, after being boiled for some time with a dilute acid, not being able to reduce Fehling's solution.

² Report of the Connecticut Agricultural Experimental Station, 1894, p. 192; also *Journ. Amer. Chem. Soc.*, 1895, p. 587.

which threw down "Precipitate No. 5." Precipitate 1 was contaminated with much colouring matter, No. 2 less so; the others were nearly colourless. Their weights were approximately; No. 1, thirteen grammes; No. 2, eight grammes; No. 3, six grammes; No. 4, five grammes; No. 5, three grammes—altogether, thirty-five grammes.

Precipitate No. 1 was further purified by solution in water, when a large amount of insoluble matter was left behind; this was removed by filtration. The filtrate from this was first dialysed in water for several days to remove salts, and then dialysed in strong alcohol until nearly the whole of the proteïds were thrown down. The precipitate thus obtained, after drying, weighed 2.11 grammes, and was almost entirely soluble in water. It had a diastatic power of 30° on Lintner's scale; its solution, when slowly heated, became turbid at 65° C., and deposited flocks at 70° C. After this coagulum had been filtered off, the liquid gave a strong biuret reaction, showing that the preparation consisted largely of proteoses.

Precipitate No. 2, after similar purification, yielded a powder almost completely soluble in water. Its solution, when heated, became turbid at 60° C., and deposited flocks at 66° C. Its diastatic power was 75°.

Precipitate No. 3, after subsequent purification, yielded 3.0 grammes of a substance almost completely soluble in water. Its solution, when heated, became turbid at 55° C., and flocculent at 60° C. The filtrate from this gave a strong biuret reaction, indicating the presence of much proteose. The diastatic power of the preparation was 222°.

Precipitate No. 4 was dissolved in water, filtered off, and dialysed for several days in water, afterwards in alcohol. Absolute alcohol was then added to the contents of the dialyser, when a precipitate weighing 4 grammes was obtained. Its solution, upon heating, became turbid at 50° C., and gave a large coagulum at 56° C. The filtrate from this gave a biuret reaction, showing that proteoses were present. This preparation had a diastatic power of 600°, and was the most powerful preparation of diastase which has ever been recorded. It was found to contain 0.66 per cent. of ash, and when subjected to analysis yielded the following results, which are corrected for ash. They show that it was a true proteïd:

	Ash-free.
Carbon	52.50
Hydrogen	6.72
Nitrogen	16.10
Sulphur	1.90
Oxygen	22.78
	<hr/>
	100.00

The amount of sulphur is somewhat large for a proteïd, but this was thought to be due to the retention of a trace of ammonium sulphate in the preparation.

Precipitate No. 5 dissolved completely in water, and, after being washed with absolute alcohol, weighed 2.87 grammes. Its aqueous solution, when

heated, became turbid at 50°C ., and yielded a small coagulum at 58°C . The filtrate from this gave a strong biuret reaction, showing that it contained much proteose. Its diastatic power was 15° .

Nature of Diastase.—The purified precipitate from No. 4 was now closely investigated. Its coagulating point agrees exactly with that of the albumin (leucosin) obtained from wheat, rye, and barley, and it has also the same chemical composition. The aqueous extracts of all these grains have a powerful diastatic action. The amount of coagulable albumin in the purified preparation amounted to 53.2 per cent. These facts strongly point to the albumin as being the active substance, yet it was not found possible to establish any numerical relation between the amount of albumin contained in a substance and its diastatic power, though in no case was a diastatically active substance ever found which was entirely free from albumin. If the activity of the preparation were solely due to albumin, then the malt ought to have shown a much higher diastatic power than it did, judging from the amount of coagulable albumin which it contained. These facts led Osborne to surmise that diastase is a compound of albumin with some other substance, presumably a proteose. Kühne, in his attempts to isolate trypsin, had been led to a similar conclusion with reference to the nature of that body. These are, however, merely suppositions, for which as yet there is no direct evidence.

Properties of Osborne's Preparation of Diastase.—Its diastatic power in comparison with other so-called pure enzymes was very great; it was able within one hour to yield with soluble starch, at the temperature of 20°C . (68°F .), 2000 times its weight of maltose, together with an undetermined amount of dextrin. After being dried over sulphuric acid and kept for six months, it lost half its diastatic power; but even then it was able in seventeen hours to produce, at 20°C ., 10,000 times its weight of maltose, besides an unknown quantity of dextrin. At 45°C . (113°F .) it produced a similar quantity of maltose in one hour, but at 50°C . (122°F .) much less maltose was formed and at 55°C . (131°F .) very little.

In a solution of the preparation, which was compared with a malt-extract having the same diastatic power, the liquefying power was found to be less; 5 c.c. of the malt-extract added to 10 c.c. of 2 per cent. starch-paste dissolved the starch completely in eight minutes, whilst 5 c.c. of the solution of the preparation required thirty-seven minutes. The malt-extract was also found to be more energetic in converting starch into bodies which gave no colour with iodine. For instance, 5 c.c. of the malt-extract added to a 10 c.c. solution of soluble starch caused the disappearance of the blue reaction with iodine in thirteen minutes, whilst it took thirty-eight minutes to arrive at the same stage with 5 c.c. of the dissolved preparation. To two portions of a starch-paste, each measuring 10 c.c., there was added to the one 5 c.c. malt-extract, and to the other the same amount of malt-extract which had been previously boiled and cooled, and to which a portion of the prepared diastase had been added, equivalent in action to that contained in the malt-extract. In the former case the starch was liquefied in seven minutes, in the latter in

fourteen minutes, whilst thirty-seven minutes were required to produce the same result with a simple aqueous solution of the preparation. The solution of the diastase in boiled malt-extract was found just as active in converting starch into bodies which gave no colour with iodine as unboiled malt-extract. Evidently, then, the difference first observed was due to the conditions of the experiment (presence of amides, mineral salts, &c.), and not to a difference in the power of the enzyme. It has been shown by Effront that one of the amides, asparagine, and certain other substances have the power of considerably accelerating the activity of diastase. This is probably on account of their influence on the reaction (acidity or alkalinity) of the medium¹ (see p. 184).

Wróblewski² investigated preparations of diastase obtained by extracting malt with 70 per cent. alcohol. He found them to consist in the main of a proteid (which constituted the active portion) and a gum-like carbohydrate (an araban). He believes that diastase is a proteid and that the enzymes belong to a special sub-group of that class of compounds, being closely related to the proteoses. He further points out that Osborne's preparations cannot have been as pure as his own as they must have contained the above-mentioned carbohydrate. Osborne³ in reply contends that his preparations were purer than Wróblewski's, in support of which he cites their greater activity.

Lintner's Method of Preparing Diastase.—The latest method prescribed by C. J. Lintner⁴ is as follows: Ground air-dried malt is extracted with 2–2½ parts of water for at least 6 hours at the ordinary temperature. The extract pressed from the grains as completely as possible is concentrated by freezing, the ice formed being freed from extract by pressing on a filter-cloth. When this operation has been repeated two or three times the solution has attained a specific gravity of 12–14° Balling, and the diastase is "salted out" by addition of ammonium sulphate. Products have been obtained in this way having a diastatic activity as high as 143°, whilst when alcohol precipitation is used, a diastatic activity of 80° is seldom exceeded.

Test for Enzymes.—Many enzyme preparations give a deep blue colour with an alcoholic solution of gum guaiacum and hydrogen peroxide, but this is due to the peroxydases and oxydases which they contain. The best way of performing this test is to prepare a 1 per cent. solution of gum guaiacum in absolute alcohol, and this should always be prepared immediately before use. Ten c.c. of this solution are placed in a test-tube, a few drops of ordinary commercial hydrogen peroxide solution added, and, if this addition causes cloudiness alcohol is added until the liquid again becomes clear. A single drop of a 0.05 per cent. solution of diastase shaken up with the mixture gives almost instantaneously a deep blue colour. So delicate is the test that the presence

¹ See Ling, *Journ. Fed. Inst. Brewing*, 1902, 8, 446; Ford and Guthrie, *Journ. Inst. Brewing*, 1905, 11, 218.

² *Ber. d. Deutsch. chem. Ges.*, 1897, 30, 2289.

³ *Ibid.* 1898, 31, 254.

⁴ *Zeit. ges. Brauw.*, 1902, 25, 365.

of oxydase in the steep water of the barley cistern can be readily detected by its agency.

Multiple Nature of Malt Diastase.—It is highly probable that the diastase of malt is not one single individual body, but a mixture of several diastases, for it has been shown that, when portions of the same solution of diastase are heated to different temperatures, and then allowed to act upon starch-paste, the products of their action are very different. Brown and Heron found that when the malt-extract, obtained by treating 200 grammes malt with 250 c.c. water, was slowly heated, a turbidity, coincident with coagulation, first appears in the fluid when a temperature of 46° C. (115° F.) was reached, and that if it was kept at this temperature for fifteen to twenty minutes, the maximum amount of coagulation for that particular temperature took place. Upon filtering off the precipitated matters and again heating the clear liquid, a fresh coagulation, reaching its maximum in fifteen to twenty minutes, commenced when a temperature slightly above 46° C. (115° F.) was reached. On repeating the process, it was found that with each successive rise in temperature a fresh coagulation took place, until, when a temperature of 95° C. (203° F.) was reached, all matter capable of coagulation by heat had been thrown down. The following table shows the quantities of coagulable matter which separate at different temperatures from 100 c.c. of malt-extract of the strength given above :

Temperature.	Grammes of Proteid Matter Coagulated.	Percentage of Total Amount.
50° C. (122° F.)	0.044	19.1
60° C. (140° F.)	0.123	34.4
66° C. (151° F.)	0.155	13.9
66° C. (169° F.)	0.186	13.4
100° C. (212° F.)	0.230	19.2
		<hr/> 100.0

They state that every stage in the coagulation of malt-extract by heat is attended with a distinct modification of its amylolytic power, and, conversely, that they had never been able to discover any modification in starch-transforming power which was not attended with distinct coagulation.

Since at 80° to 89° the diastatic power of malt-extract is completely destroyed, and this is the temperature at which nearly the whole of the coagulable proteids were thrown down, they concluded that the diastatic power was a function of the soluble proteids, and was not due to a distinct agent. They found that, when malt-extract is filtered under slight pressure through a porous earthenware battery cell once or twice, all the proteids were retained within the cell, and that the filtered liquid was as completely deprived of its diastatic power as if it had been boiled.

Barley Diastase.—The fact that ungerminated grain contains an amylolytic enzyme has been known for a long time; its presence in the gluten of wheat had been detected as early as 1812 by Kirchoff. This fact had been lost sight of until quite recent times, when experiments were made

on ungerminated grain, and its diastatic power ascertained by Lintner's method, in which soluble starch is used instead of starch-paste. It was then found that the diastatic power of ungerminated grain often equalled, and sometimes surpassed, that of ordinary malt. In its action on starch barley diastase differs widely from malt diastase; its power of liquefying gelatinised starch is very much inferior to that of malt diastase; the latter exerts as much liquefying action on a solution of gelatinised starch in eight minutes as an equivalent quantity of barley diastase does in twelve hours. It is also unable to corrode starch granules; this and the power of liquefying gelatinised starch, according to Brown and Morris, appear to go hand in hand. Lintner and Eckhardt¹ found considerable differences in the action of the two diastases at various temperatures. Thus the optimum temperature of barley diastase was from 40° to 50° C., that of malt from 45° to 55° C.; the former was as active at 4° C. (39° F.) as the latter was at 14.5° C. (58° F.).

Action of Ungerminated Barley Diastase on Starch.—An important paper on this subject was read before the Chemical Society by J. L. Baker in 1902.² Baker finds that when the diastase from raw barley is allowed to act on a solution of soluble starch at 50° C., hydrolysis proceeds until at the end of an hour to an hour and a half the constants of the dissolved matter are $[\alpha]_{D_{3.93}} 160 - 165^\circ$ and $R_{3.93}^* 60 - 65^\circ$. After this the reaction is a comparatively slow one, and if allowed to continue for 96 hours the reducing power is only increased to $R_{3.93} 70$, the iodine reaction of the mixed products being still blue. An examination of the products after the reaction had proceeded for 1½ to 2 hours, showed that a dextrin and maltose were the sole products. After 24 hours evidence of the presence of glucose was obtained, the amount of this sugar apparently increasing in proportion to the time of conversion.

The dextrin isolated by precipitation with alcohol from the products of conversion gave a blue iodine reaction, a specific rotation of $[\alpha]_{D_{3.93}} 190 - 195^\circ$, and a reducing power of $R_{3.93} 0.55 - 1$. The alcoholic solutions from which the dextrin was precipitated were collected and found to contain maltose, glucose (if the conversion period was prolonged), but no trace of any dextrin of less complexity than that described above.

Barley diastase acts on the above-mentioned dextrin very slowly, the constants of the dissolved matter after 90 hours' action at 45-50° being $[\alpha]_{D_{3.93}} 160^\circ$ and $R_{3.93} 32$. The mixed products, which give a blue iodine reaction, consist of maltose, unaltered dextrin, and glucose. The presence of glucose could be detected in 18 hours, but after 90 hours the amount had only slightly increased. The action of malt diastase on this dextrin was more vigorous. After 18 hours at 55° C. the mixed products which no longer gave the blue iodine reaction, had the constants $[\alpha]_{D_{3.93}} 149.3^\circ$ and $R_{3.93} 50 - 51$.

¹ *Zeitsch. für Brau.*, 1886, p. 261.

² *Journal of the Chemical Society, Trans.*, 1902, 1177.

* The symbol R denotes the cupric reducing power expressed in terms of percentage of apparent maltose on the dry matter in solution.

These products consisted of a mixture of achroo-dextrins and a considerable proportion of glucose. Since barley diastase is without action on maltose the glucose found in the products from the prolonged action of barley diastase on soluble starch must owe its origin to the hydrolysis of the dextrin.

Contrary to the statements of certain other observers, barley diastase was found by Baker to completely liquefy starch-paste in 2–3 hours at 50° C., the products consisting of the above-described dextrin and maltose.

The dextrin obtained by the action of barley diastase on soluble starch or on starch-paste differs markedly from Nægeli's "amylodextrin" which was investigated by Brown and Morris in 1889. In consideration of its general behaviour to the parent substance, starch, Baker proposes to term the new dextrin " α -amylodextrin."

Glucase.—An enzyme which is unable to act upon gelatinised starch, but which slowly converts soluble starch, more readily dextrin or maltose, into glucose. It has been said to be present in steeped barley, and also in malt, by several observers, who state that it exists there partly in a soluble and partly in an insoluble condition. Other authorities have been unable to detect the presence of this enzyme in either barley or malt. Glucase appears, from the researches of Géduld,¹ to be a normal constituent of maize. He prepared the enzyme by extracting ground maize with water, and precipitating the enzyme from the extract thus obtained with alcohol. The action of this enzyme on dextrin and maltose is most energetic at temperatures between 50° and 60° C. (122° to 140° F.); at 60° C. the action becomes slower, and it is completely arrested at 70° C. (158° F.).

Laccase.—An enzyme, found by Bertrand² in the tree which yields lac, which possesses the power of absorbing oxygen from the air, and of evolving carbon dioxide. This action so closely resembles respiration that it has been thought to stand in some definite relation to this process. Oxidising enzymes exist in malt, which effect the transformation of a portion of the carbohydrates into water and carbon dioxide.

THE ENZYMES OF YEAST.

The cultivated yeasts contain several enzymes; the longest known of these is invertase, which possesses the power of inverting cane-sugar. In order to obtain invertase, A. Meyer³ allowed pressed yeast to stand for some time under alcohol, to kill the cells. The yeast was then freed from alcohol and triturated with sand and water to rupture the cells; the aqueous extract thus obtained was filtered off and alcohol added. The enzyme was precipitated in an impure condition, and subsequently dried over sulphuric acid in a vacuum. C. O'Sullivan allows pressed yeast to stand in a cool place until the mass liquefies; the liquid portion is then filtered off, and the enzyme precipitated with alcohol and dried, as in the foregoing process.

¹ *Woch. für Brau.*, 1891, p. 545.

² *Compt. rend.*, 120, 266.

³ Meyer, *Die Lehre v. d. Fermenten*, p. 64.

The most favourable temperature at which invertase exerts its action on cane-sugar is, according to Kjeldahl,¹ for top fermentation yeast 55° to 57° C. (131° to 135° F.). According to J. O'Sullivan,² invertase cannot pass out of the yeast-cell by exosmosis; consequently, the inversion of sugar, under normal conditions, takes place within the yeast cell.

Yeast Maltase.—In 1866 Bourquelot³ came to the conclusion that maltose suffered hydrolysis before it was fermented by yeast, and noticed that when yeast was allowed to remain for several days in a solution of maltose saturated with chloroform, the optical rotatory power of the solution was diminished, presumably through the formation of glucose. Lintner,⁴ in studying the fermentation of isomaltose, surmised that both this sugar and also maltose were converted into glucose by the invertase of the yeast before being fermented. E. Fischer⁵ prepared, by extracting 1 part of dried yeast with 15 parts of water at 30° to 35.3° C. (86° to 95° F.), a solution which had the power of hydrolysing maltose; but he was unable to obtain the same result with ordinary commercial invertase. Lintner⁶ expressed the view that the maltose inverting ferment was distinct from invertase. He obtained the most powerful action on maltose by adding powdered yeast, which had been dried at a temperature of 40° C. (104° F.), to a solution of this sugar. The aqueous extract from the same powdered yeast exhibited much less activity, and the substance obtained by precipitation with alcohol still less. E. Fischer⁷ showed that the enzyme was already present in the yeast-cell, and was not formed during the process of drying, for on allowing ordinary yeast to act upon solutions of maltose which contained either 2 per cent. of thymol or 3 per cent. of toluene (added to arrest alcoholic fermentation), considerable quantities of glucose were formed. So long, therefore, as yeast is moist and fresh, the hydrolytic action takes place within the cells.

Reversible Zymohydrolysis.—A. Croft Hill has shown⁸ that the hydrolysis of maltose to glucose by yeast maltase is incomplete and is hindered by the presence of glucose, both these effects being more marked the more concentrated the solution. He finds that when a concentrated solution of glucose is treated with maltase the optical activity increases and the cupric reducing power decreases by amounts consistent with the assumption that some of the glucose is converted into maltose. Moreover, a phenylsazone soluble in hot water was prepared from the product which had the percentage composition and ordinary physical characteristics of phenylmaltosazone. It had, however, a lower melting-point (186° C.) than phenylmaltosazone. Starting with a solution of maltose of the same concentration as that of the glucose, the same point was reached, so that it appeared that the same state of equilibrium is attained no matter whether a solution of glucose or of maltose

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² *Proceedings of the Chemical Society*, 1892, p. 124.

³ *Journ. de l'Anatomie et Phys.*, 1866, p. 180.

⁴ *Zeit. f. Brau.*, 1892, p. 107.

⁵ *Ber. d. Deutsch. chem. Gesells.*, 1894, 27, 2986.

⁶ *Zeit. f. Brau.*, 1894, p. 415.

⁷ *Ber. d. Deutsch. chem. Gesells.*, 1895, 28, 1429.

⁸ *Journ. Chem. Soc., Trans.*, 1898, 634-658.

is acted upon by the enzyme provided the concentration is the same in both cases. O. Emmerling¹ confirmed Croft Hill's observation that yeast maltase exerts a condensing action on glucose, but he contends that "isomaltose," (not maltose) is formed together with reversion dextrins. The "isomaltose" was isolated from the product after allowing it to ferment with ordinary yeast. Further papers of a controversial nature followed these, and Emmerling² has since extended his observations on the synthetic action of yeast-maltase. This enzyme converts amygdalin of bitter almonds into glucose and the glucoside of mandelic nitril. By acting upon these twolast-mentioned compounds with yeast-maltase at 35° C. for three months a small amount of amygdalin was formed.

The latest work on this subject is by Croft Hill.³ Dealing with the action of yeast-extract containing maltase on maltose or on glucose he finds that when equilibrium is attained two isomeric hexabioses are present, one which he has isolated being denoted *revertose*, whilst the other, although not isolated, has the properties of maltose. Revertose, which has not been obtained in detached crystals, has a specific rotatory power $[\alpha]_D + 91.5^\circ$. It possesses a sweet taste, is not fermented by all yeasts which ferment maltose, although it seems to be slowly attacked by some; Hill thinks it probable that the sugar may be present in certain beers. Synthetical products are also formed by the action of Taka diastase and of pancreatic enzymes on glucose.

Melibiose.—It has long been known that the top fermentation yeasts were unable to ferment more than a third part of melitriose (raffinose), but that the bottom fermentation yeasts were able to ferment this sugar completely. From this it would appear that low yeasts contain an enzyme which is capable of inverting melibiose (see p. 50) which the top yeasts do not possess. That this is actually the case was demonstrated experimentally by A. Bau,⁴ who called the enzyme "melibiase." Melibiase, like yeast-glucase, is a comparatively insoluble body.

Other Enzymes.—It is evident that other enzymes are contained in such yeasts as the Froberg varieties or the Schizo-sacc. Pombe, since these are able to hydrolyse some of the higher malto-dextrins. It has been shown by E. Fischer that *Monilia candida*, which was supposed to be able to ferment cane-sugar without previous inversion, contains in its protoplasm an insoluble enzyme which can effect the hydrolysis of cane-sugar.

THE CHEMICAL COMPOSITION OF YEAST.

Since yeast, under the older theories, was regarded as a putrefying substance which merely communicated a portion of its own internal motion to the fermentable substance, and neither gave off anything from itself to the fermenting liquid nor took up anything from the substances dissolved in that liquid, a detailed knowledge of its composition was comparatively a matter of

¹ *Ber. d. Deutsch. chem. Gesells.*, 1901, **34**, 600-605. ² *Ibid.*, 1380 and 2185.

³ *Journ. Chem. Soc., Trans.*, 1903, **83**, 578.

⁴ *Woch. f. Brau.*, 1895, p. 24.

indifference; consequently, the older analyses of yeast were mostly confined to a determination of its elementary composition. But when yeast was found to be a living organism, that not only derived its nutriment from the bodies contained in the fermenting liquid, but which also added its own excretions to that liquid, then it became necessary to extend this simple form of analysis, and to isolate and determine as far as possible the nature and composition of the various groups of substances which entered into its composition. In this way chemists endeavoured to study the relations subsisting between the structural compounds of yeast and of those substances which form its nutriment, much in the same way as an agricultural chemist studies the relation between the constituents of plants and those of the soil. Numerous investigations have been made in this direction, but the subject is one of considerable intricacy, for the examination of yeast, in addition to the inherent difficulties of this kind of analysis, presents some obstacles which are peculiarly its own. Yeast is always more or less contaminated with foreign impurities, and before it can be analysed with any degree of exactitude these must be removed. It is generally sought to effect this object by washing with water, but in so doing some of the soluble constituents of the yeast are invariably dissolved and removed by the wash-water, whilst at the same time water is absorbed by the yeast, the result being an apparent lowering of the amount and alteration in the proportions of its various constituents. Moreover, yeast is a body whose chemical composition is in a perpetual state of change, and, consequently, analyses of the same yeast made at different times yield dissimilar results. The varieties of yeast which have invariably been chosen for investigation in this way are the high and low fermentation forms of the *Saccharomyces cerevisiae*. Little is known about the composition of other varieties of yeast, such as the *S. ellipsoideus*, *S. apiculatus*, &c., or of the bacteria.

The Elementary Composition of Yeast.—The yeast-cell consists of water and solid matter, the former constituting about 80 per cent. of its weight.¹ The solid substance consists of carbon, hydrogen, oxygen, nitrogen, and sulphur, together with a small quantity of mineral substances (ash). The results of a number of elementary analyses of the dry substance of yeast, which was in each case freed from foreign substances by washing, are appended :

	Mitscherlich.	Schlossberger.		Dumas.	Wagner.		Mulder.
	High Yeast.	High Yeast.	Low Yeast.		High Yeast.	Low Yeast.	High Yeast.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Carbon . . .	47.0	49.9	48.0	50.6	49.8	44.4	50.8
Hydrogen . . .	6.6	6.6	6.5	7.3	6.8	6.0	7.2
Nitrogen . . .	10.0	12.1	9.8	15.0	9.2	9.2	11.1
Oxygen . . .	35.8	31.4	35.7	37.1	34.2	35.8	?
Sulphur . . .	0.6	?	?				

¹ Pressed yeast contains between 70 and 80 per cent. of water.

These analyses, as might be expected, exhibit considerable differences, but the most striking feature of the composition of yeast is the large amount of nitrogen which it contains when compared with that found in other plants,¹ and even in those belonging to the same genus. The common mushroom (*Agaricus campestris*), the dry substance of which has been found to contain from 7.26 per cent. to 7.33 per cent. of nitrogen, approaches it most nearly in this respect.

According to the analyses of yeast by Hayduck, the amount of nitrogen it contains may vary from 4 to 10 per cent., and this quantity may be altered at will by growing the yeast in a liquid poor or rich in assimilable nitrogenous food.

Wijsmann² found that the amount of nitrogen contained in yeast is in a constant state of variation. When 10 grammes of yeast, which contained 7.09 per cent. of nitrogen (reckoned on the dry substance), were added to a litre of malt wort, the nitrogen increased in the course of an hour to 9.9 per cent., in two hours it had slightly decreased to 9.6 per cent., and in three hours it was 9.55 per cent. In another experiment where yeast was added to ordinary service-water containing in solution 0.7 per cent. of peptone, the 6.36 per cent. of nitrogen originally present in the yeast increased in ten minutes to 7 per cent., in fifty minutes to 7.08 per cent., and in eighty-five to 7.22 per cent. In further experiments yeast was added, in the one case to service-water containing in solution 0.25 per cent. of asparagine and 0.1 per cent. of potassium sulphate; in the other, to a similar solution in which 3 per cent. of cane-sugar had been dissolved. The amount of nitrogen of the original yeast (6.88 per cent.) varied in the following manner:

Hours.	Minutes.				Without Sugar. Per Cent.	With Sugar. Per Cent.
2	21	.	.	.	8.10	9.27
3	5	.	.	.	7.91	9.24
3	40	.	.	.	7.43	9.55
5	0	.	.	.	7.45	9.24

Curiously, when yeast was immersed in a solution of acid ammonium phosphate, it quickly absorbed its maximum quantity of nitrogen, and this did not again diminish, as was the case when yeast was placed in malt wort. Wijsmann conjectures that yeast, at the commencement of fermentation and before it exercises its reproductive powers to the fullest extent, stores up a large quantity of nitrogenous material. It may be that this material is either assimilated by the protoplasm in preparation for budding, and, as budding proceeds, gradually returns to its original quantity, or that the nitrogenous bodies are at first simply absorbed in an unaltered condition, and are afterwards metamorphosed by the protoplasm. He concludes, from other experiments, that acid ammonium phosphate is not absorbed unchanged. In

¹ The nitrogen of the dry substance of ordinary plants ranges from 0.3 per cent. to 4.5 per cent. Ebermayer, *Phys. Chem. d. Pflanzen.*, i. 47.

² *Woch. f. Brau.*, 1891, p. 1887.

those cases where yeast was compelled to obtain its nitrogen from peptone, asparagine, or acid ammonium phosphate, the increase in its nitrogen was never so large as when it was grown in malt wort.

The Mineral Constituents of Yeast.—The amount of ash found in yeast by different observers varies from 2.5 per cent. to 9.0 per cent. The following table contains a number of analyses of the ash of yeast by different chemists :

	Mitscherlich.		Bull.	Liebig.	Béchamp.
	High Yeast.	Low Yeast.	High Yeast from Weissbier.		
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Phosphoric acid . . .	53.9	59.4	54.7	48.5	53.7
Potassium	39.8	28.3	35.2	30.6	28.6
Soda	...	...	0.5	...	1.4
Magnesia	6.0	8.1	4.1	4.2	5.2
Lime	1.0	4.3	4.5	2.1	2.4
Iron oxide	...	...	0.6	...	5.0
Sulphuric acid ¹ . . .	...	...	...	...	5.7
Hydrochloric acid . .	...	...	0.1	...	...

Though the composition of the ash of yeast, as portrayed by the above analyses, varies considerably, yet these analyses all show that the most abundant of its constituents are phosphoric acid and potash, substances which have been proved to be indispensable in the nutrition of all the higher plants. The next body which occurs in fairly large quantity is magnesia, and this is also an indispensable substance. Lime and iron appear in smaller quantities (the large amount of iron shown in the analyses by Béchamp was probably due to contamination), and it is doubtful if the latter substance is an absolutely necessary constituent of yeast. Nägeli ² considers that the potassium salts are dissolved in the cell-juice, whilst those of the alkaline earths are contained in the more solid tissues. In all the lower fungi it is supposed that the potassium bases are united with phosphoric or an organic acid, the potassium being in combination with phosphoric acid in the cell-juice as hydrogen-dipotassium phosphate (HK_2PO_4), a portion of the alkaline earths are assumed to exist as phosphates in the plasma, whilst other portions are deposited as compounds of organic acids (oxalic, &c.) in the cell-membrane.

Differentiation of the Different Groups of Compounds occurring in Yeast.—The solid matter of yeast may be divided into two principal groups, the nitrogenous and the non-nitrogenous, each group being subdivided into several members.

¹ Phosphoric acid acts under certain circumstances, as, for instance, in the case of the ignition of a yeast ash, as a more powerful acid than sulphuric acid. The latter may consequently be partially or completely driven off, and this is the reason why its presence has not been detected in a large number of analyses. Béchamp took special precautions to avoid loss from this cause.

² *Sitz. d. bayer. Acad.*, 1889, Part iii.

Yeast Cellulose.—By the almost universal consent of all observers, the enveloping membrane of the-yeast cell, like that of all other vegetable cells, consists of a variety of cellulose. Its amount has been estimated as forming from 18.5 per cent. (*Pasteur*) to 32 per cent. (*Payen*) of the dry yeast substance, the wide difference in the amounts given by various observers being undoubtedly due to the extreme ease with which cellulose undergoes alteration. It is very readily converted into sugar, an action which appears to take place naturally sometimes, as in the spontaneous fermentation which occurs in yeast when it is deprived of nutriment. Schlossberger, by treating yeast alternately with solution of caustic potash and acetic acid until nothing further was dissolved, and washing with water, obtained a residue of the following composition :

	Per Cent.
Carbon	44.9
Hydrogen	6.7
Nitrogen	0.5
Ash	1.1

This corresponds with the composition of an impure cellulose.

Pasteur, on treating yeast with dilute sulphuric acid found an amount of sugar produced which corresponded to 18.5 per cent. of cellulose. It is thought by some observers that the cellulose of yeast and of the rest of the fungi differs markedly in some of its properties from that of other plants, since it is insoluble in cuprammonium hydrate solution, and is readily attacked by dilute acids. Dreifuss,¹ on treating several of the fungi with dilute acid, alkali, alcohol, and ether, and then with concentrated caustic alkali at a temperature of 180° C., obtained a residue which, on boiling with a dilute acid, yielded glucose. This substance, which he considered to be the ordinary "plant cellulose," was found in the common mushroom, in *Bacillus subtilis*, and in *Aspergillus glaucus*. Winterstein² obtained from certain fungi, on prolonged treatment by Schultz's method, a cellulose which differed considerably from ordinary plant cellulose. It only gave the blue or violet reaction with iodine and strong sulphuric acid after some time, and was but partially soluble in cuprammonium hydroxide. It contained from 1.64 to 3.94 per cent. of nitrogen, which could not have been derived from proteïds. On hydrolysis with 70 per cent. sulphuric acid it yielded 65.95 per cent. of glucose, there being formed at the same time 12.52 per cent. of acetic acid. Gibson³ was unable to obtain the crystalline variety of cellulose from fungi which he had obtained from other plants. Liebermann and V. Bitto⁴ obtained yeast cellulose in a pure state by digesting yeast for several hours on the water-bath with a mixture of equal parts of hydrochloric acid and water, to which was added a small quantity of potassium chlorate. The residue was well washed, boiled for half-an-hour with 1.25 per cent. solution of acetic acid, and finally boiled with a 1.25 per cent. solution of potash. It gave the ordinary reaction of cellulose with iodine and zinc chloride solution.

¹ *Zeit. f. phys. Chemie*, **18**, 358.

² *Ber. d. Deutsch. chem. Ges.*, 1893, **26**, 3098.

³ *La Cellule*, 1893.

⁴ *Centralblatt f. Physiologie*, vii. 857.

Yeast Mucilage.—Nägeli obtained in the analysis of yeast 37 per cent. of cellulose and mucilage. This latter substance is one which occurs in great abundance in the seeds of certain plants, such as linseed, quince, &c. It shows its near relationship to cellulose in being coloured violet by iodine and sulphuric acid. On hydrolysis with dilute acids it yields a reducing sugar which is probably a mixture of hexoses and pentoses. The mucilaginous coating discovered by Hansen, which, under certain conditions, forms around yeast-cells, is probably of the same nature.

Yeast Gum.—Hessenland¹ obtained a gum on boiling yeast with lime, having the same formula as Scheibler's dextran, $C_6H_{10}O_5$, but which does not seem to be identical with it. On hydrolysis with dilute sulphuric acid this gum yielded chiefly d-mannose and a little glucose.

Salkowski² obtained, by acting on yeast with 3 per cent. solution of caustic potash, and heating the resulting liquid with Fehling's solution, a copper compound of a gum. This was dissolved in hydrochloric acid, precipitated by alcohol, washed with alcohol and ether, and dried. In this way a fine white powder was obtained, which in some respects resembled gum arabic. It had a composition of $C_{12}H_{22}O_{11}$, and an optical rotatory power of $[\alpha]_D + 90.1^\circ$. Acids converted this gum, of which the dry substance of yeast contained about 7 per cent., into a feebly dextro-rotatory sugar capable of undergoing fermentation. Salkowski considers that the gum prepared by Hessenland's method is a mixture of several substances.

The Fat of Yeast.—Yeast contains a certain amount of fat, which, when the cells are examined under the microscope, is seen in the form of bright strongly refractive spots. Nägeli estimates its quantity at about 5 per cent., but considers that its amount varies, young cells being poor, older ones rich in fat.

Kulish³ carefully investigated the composition of yeast-fat, and found it to consist chiefly of phytosterin and a glyceride of myristic acid; the glyceride of another acid, the nature of which he was unable to determine, also occurs in small quantity. The formation of fat in yeast takes place most rapidly when yeast reproduction proceeds most actively, and when yeast is supplied with an abundance of oxygen (Nägeli).

Glycogen.—This carbohydrate, which has been long known as one of the normal constituents of the animal liver, was first found in yeast by Errera.⁴ In a pure condition it is a white powder, having the composition $C_6H_{10}O_5$, readily soluble in hot water, with which it forms an opalescent solution which does not reduce Fehling's solution. Glycogen is readily converted into glucose by boiling with a dilute acid; both the enzymes ptyalin and diastase convert it into a mixture of maltose, glucose and dextrin.

An aqueous solution of glycogen is coloured reddish-brown by iodine solution, a reaction by which the presence of glycogen may be readily detected

¹ *Zeit. Rübenzucker Ind.*, 1892, p. 671.

² *Ber. d. Deutsch chem. Ges.*, 27, 495.

³ *Weinbau u. Weinhandel*, 1891, p. 250.

⁴ *Compt. rend. de l'Acad. des Sciences*, 1885, 101, 253.

in yeast-cells. Laurent,¹ who studied the formation of this substance in yeast, found that it was produced as a reserve material, and that it might sometimes amount to 32.6 per cent. of the dry yeast substance, and that it could be formed by yeast from the salts of either lactic or succinic acid, from ammonium succinate, glycerin, glucose or cane-sugar, dextrin, asparagine, &c. According to Max Cremer,² fresh yeast-cells, when treated with a dilute solution of iodine in potassium iodide, exhibit the glycogen reaction in a marked degree, but yeast-cells which have been starved, such as those which have undergone spontaneous fermentation, are only coloured pale yellow. If the starved cells be placed in a solution of glucose, fructose, or cane-sugar, the glycogen reaction can be again detected. He was unable to observe the formation of glycogen in similarly starved cells when these were introduced into a solution of one of the pentose sugars, or of glycerin, or of milk-sugar, all of which substances are unfermentable by ordinary yeast. A. Harden and W. J. Young³ have compared glycogen prepared from rabbit's liver, oysters, and yeast, and conclude that they are one and the same substance. The subject is further dealt with in a subsequent part of this work

The Nitrogenous Constituents of Yeast.—As with the nitrogenous bodies of all other organisms, much uncertainty prevails with regard to the nature and composition of those of yeast, and though many attempts have been made from time to time to increase our knowledge in this direction, very much yet remains to be done.

Schlossberger⁴ attempted to isolate the nitrogenous compounds of yeast by treating it with a dilute solution of caustic alkali, and neutralising the filtered liquid thus obtained with an acid. A substance came down in the form of a flocculent precipitate; it contained no ash, and had the following composition :

	Per Cent.
Carbon	55.5
Hydrogen	7.5
Nitrogen	13.9
Sulphur	0.0

It is to be assumed that the remainder was oxygen. This substance, from the small amount of nitrogen which it contains, does not appear to be a normal albuminoid; it corresponds closely in composition with a body found by Schützenberger⁵ amongst the products of the hydrolysis of albumin by dilute sulphuric acid, and called by him "hemi-protein," but which is now known as "anti-albumin." Mulder, by treating yeast with acetic acid, filtering off the resulting liquid and precipitating with ammonium carbonate, obtained a body more nearly resembling albumin in composition :

	Per Cent.
Carbon	53.3
Hydrogen	7.0
Nitrogen	16.0

¹ *Botanische Zeit.*, 48, 719.

² *Zeit. f. Biologie*, 31, Part ii.

³ *Journ. Chem. Soc., Trans.*, 1902, 81, 1224.

⁴ *Ann. d. Chem. u. Pharm.*, 11, 199.

⁵ *Bull. Soc. Chim.*, 23 and 24.

According to the investigations of Nägeli and Löw,¹ the plasma of a young yeast (which contained nearly 12 per cent. of nitrogen) yielded about 75 per cent. of proteïds and about 2 per cent. of peptone. They state that during the gradual series of changes which yeast-cells undergo previous to dying, the whole or nearly the whole of the proteïds are transformed into peptones.² They give the following proximate analysis of a sample of yeast which contained 8 per cent. of nitrogen :

	Per Cent.
Cellulose and mucilage	37
Proteïds { Albumin	36
{ Substance resembling gluten-casein	9
Peptone	2
Fat	5
Ash	7
Extractive matters	4

The 4 per cent. of extractive matters were not precipitable by lead acetate; they contained a peptone-like body, small quantities of invertase, leucine, glucose, and still smaller quantities of glycerin, succinic acid, guanine, xanthine, sarkine, alcohol, and probably traces of inosite.

Nucleïn.—This proteïd, which seems to be a normal constituent of the nuclei of cells, has been isolated from yeast by treating it for a short time with a 5 per cent. solution of caustic potash. The resulting solution is filtered into dilute hydrochloric acid, the precipitate which forms is washed first with dilute hydrochloric acid, then with alcohol, boiled several times with alcohol, and finally dried over sulphuric acid. Thus obtained, it forms a white amorphous powder, insoluble in water and dilute acids, and readily soluble in dilute alkalis. When boiled for a considerable length of time with water, the greater portion of it is transformed into phosphoric acid and xanthine bodies. When fused with sodium carbonate and nitrate, it yields a mass containing phosphoric acid in large amount. Its probable composition, according to Miescher, is $C_{29}H_{49}N_9P_3O_{22}$. Nucleïn is distinguished from the rest of the proteïds by the large amount of phosphorus which it contains (9.59 per cent.), and by the extreme resistance which it offers to the hydrolysing action of pepsin. It gives an indistinct xantho-proteïc reaction, but gives no reaction with Millon's reagent. According to recent views, nucleïn is a compound of nucleïc acid (a compound of albumin and phosphoric acid) with one or more of the xanthine bodies.

Specific Gravity of Yeast.—Guichard³ found the specific gravity of yeast to be from 1.180 to 1.183 at a temperature of 16° C. (61° F.).

Products of the Putrefaction of Yeast.—These are of practical interest to the brewer, since putrefaction of yeast occasionally takes place in beer, the composition and properties of which it may affect if, as is sometimes the case, the beer is allowed to remain for a long time in contact with putrefying yeast. Muller⁴ obtained, on adding calcium hydroxide to liquid

¹ *Sitzb. d. bayer. Acad.*, 1878, Part ii.

² By peptones is meant in this case substances precipitated by lead acetate.

³ *Bull. Soc. Chim.*, 1894, 11, 230.

⁴ *Journ. f. prakt. Chemie*, 70, 65.

putrefied yeast, a precipitate which, when distilled with dilute sulphuric acid, yielded a number of volatile acids. These were purified by conversion into their barium, sodium, or silver salts. Acetic acid was found in large, butyric acid in moderate quantity, also in smaller amount caprylic acid, together with traces of the higher acids of the same series. On distilling the liquid from which the precipitate had been obtained, collecting the distillate in dilute hydrochloric acid, and purification of the salts thus obtained, he was able to identify ammonia, trimethylamine, ethylamine (probably), amylamine, and caprylamine. This probably explains the formation of the methylamine sometimes found in wine. Lactic acid was also present, and this is not surprising, as yeast is often contaminated with lactic acid bacteria. Tyrosine crystallised out in chalk-like masses, and leucine was also found; both of these bodies are found amongst the products of the hydrolysis of albumin by dilute sulphuric acid. Bodies of a fusel-oil-like nature were also found, the principal one being amylic alcohol, and this lends some probability to the view held by some that fusel-oil is a body produced by the yeast when in a moribund condition.

THE ACTION OF DIASTASE ON STARCH.

This is a subject of the most profound importance to the brewer, for the products of the action of diastase on starch differ widely, according as the circumstances vary under which these two bodies are allowed to interact, and the products formed in this way have an immense influence on the character of the finished beer.

The subject is one which has attracted an enormous amount of attention for a long series of years, and which has led to many differences of opinion amongst observers. Even at the present time these are far from being reconciled; and although our knowledge in recent years has made considerable progress, much still remains to be explained and established with certainty.

In the opinion of the earliest observers, the transformation which took place when diastase acted upon gelatinised starch was that the starch molecule simply united with a molecule of water, and formed glucose according to the following equation:

Later on it was found that another body, dextrin, was invariably produced in all starch transformations; and for a long time it was considered that the substance first produced was dextrin, this becoming afterwards converted into glucose. According to this view, dextrin is a body intermediate between starch and glucose.

In 1860 Musculus¹ brought forward evidence to show that this was not

¹ *Ann. Chim. Phys.* [3] 55, 203.

the case, but that in all starch transformations, be they effected either through the agency of diastase or of dilute acids, dextrin and sugar were produced simultaneously.

This view, though it was strongly controverted at the time it was brought forward, is the one which has survived, and which, in an extended form, has been found by the majority of workers on the subject to afford the best explanation of the observed phenomena.

Musculus was led to believe that the conversion invariably took place according to the following equation :

that is, from every three molecules of starch two molecules of dextrin¹ and one molecule of glucose were formed.

Other observers, however (*Payen, Guerrin-Varry, Balling, Dubrunfaut*), in their experiments, obtained the two bodies dextrin and glucose in proportions widely differing from those which are required by the above equation.

In 1870 Schwartz² pointed out that these discrepancies were caused by the observers not having taken into account the different temperatures at which the various starch conversions had been effected. He found that if the action were allowed to proceed at any temperature between 0° to 30° C. (32° to 86° F.), the products of the transformation were in the ratio of one molecule of dextrin to one molecule of glucose, according to the following equation :

But if the process were conducted at a temperature above 60° C. (140° F.), two molecules of dextrin to one molecule of sugar were produced, thus :

So far it had been considered that in all starch transformations but one dextrin was produced, but in 1871 Griessmayer³ concluded from his experiments that two dextrins were formed; these he designated "Dextrin I. and II." The following year C. O'Sullivan⁴ confirmed this, and called the two dextrins "α" and "β." In this same year Brücke⁵ gave them the names "erythrodextrin" (ἐρυθρός, red) and "achroodextrin" (ἄχρους, without colour), since the first was coloured red by iodine, and the latter gave no colour with that reagent, and this nomenclature has been retained to the present day. O'Sullivan carefully examined these two dextrins in the following manner :

¹ The complexity of the starch and dextrin molecules at that time was regarded as corresponding with the formula $\text{C}_6\text{H}_{10}\text{O}_5$, which was, of course, an erroneous assumption.

² *Journ. f. prakt. Chemie* [2], 1, 212.

³ *Annalen*. 160. 40.

⁴ *Journ. Chem. Soc.*, 1872, 25, 579.

⁵ *Wien. Acad. Bericht* [3]. 65. 126.

Starch conversions were made in some cases by the agency of diastase, in others by that of dilute sulphuric acid; the action being stopped sometimes when the conversion gave a deep brownish-red colour with iodine, at other times when this reagent had ceased to give a colour. In those cases in which the conversion had been effected with diastase, the further action of the enzyme was arrested by simply boiling the solution; in those in which sulphuric acid had been employed, by removing the acid by barium hydrate.

After the conversion the solutions were in each case evaporated to a small bulk, and strong alcohol (sp. gr. 0.830)¹ added in considerable quantity. The precipitate of impure dextrin thus obtained was further purified by being dissolved in water, and again precipitated with alcohol, the re-solution and re-precipitation being repeated in some cases as many as thirty times. In this way four preparations of dextrin were obtained, having the following optical rotatory and reducing powers:

<i>a.</i> $[\alpha]_D$	=	+204°	K=8.8		<i>c.</i> $[\alpha]_D$	=	+204°	K=9.0
<i>b.</i> $[\alpha]_D$	=	+205°	K=8.5		<i>d.</i> $[\alpha]_D$	=	+198°	K=9.0

a was obtained by the action of malt-extract; *b* by that of dilute sulphuric acid; *c* by that of oxalic acid; *d* was prepared with dilute sulphuric acid, the action in this case being allowed to continue over eight hours. It was evident that in all these cases a considerable quantity of some substance having a reducing action upon Fehling's solution was present, and in order, if possible, to still further remove this, solutions of each of the above preparations were dissolved in water, and submitted to fermentation with yeast. When the fermentation had ceased, the liquid was filtered off, reduced to small bulk, and alcohol added. The dextrin thus obtained was several times dissolved in water, and re-precipitated by alcohol. The last precipitate, after being washed twice or thrice with alcohol, was pressed between folds of filter-paper, and finally dried in a vacuum over sulphuric acid. The dextrins obtained in this way were white, semi-crystalline, highly hygroscopic powders, not perceptibly soluble in cold alcohol of specific gravity 0.82, but readily soluble in water; and when one of these dehydrated dextrins was dissolved in water, its solution was accompanied with evolution of heat. The following table exhibits the optical rotatory powers and reducing powers of eight preparations made in this way:

<i>e.</i> $[\alpha]_D$	=	+213.0°	K=2.03		<i>i.</i> $[\alpha]_D$	=	+212.7°	K=1.40
<i>f.</i> $[\alpha]_D$	=	+212.0°	K=2.20		<i>j.</i> $[\alpha]_D$	=	+213.0°	K=1.15
<i>g.</i> $[\alpha]_D$	=	+212.7°	K=1.24		<i>h.</i> $[\alpha]_D$	=	+213.5°	K=0.80
<i>h.</i> $[\alpha]_D$	=	+213.1°	K=1.20		<i>l.</i> $[\alpha]_D$	=	+214.0°	K=1.03

e, *f*, and *g* are from the product of the action of diastase on starch-paste, the transformation being stopped when a portion of the solution, after being allowed to become cold, gave a reddish-brown colour with iodine (erythro-dextrin). *h* and *i* were prepared in the same way, the action being arrested

¹ This would contain 87 per cent. of alcohol and 13 per cent. of water by weight.

when iodine ceased to give any coloration (achroo-dextrin). *j* was obtained from a transformation effected by dilute sulphuric acid, the acid being removed when iodine gave a reddish-brown colour (erythro-dextrin). *k* was produced similarly to *j*, with the exception that the acid was removed when iodine ceased to give any colour (achroo-dextrin). *l* was obtained by the action of oxalic acid, the action being stopped when the solution gave a reddish-brown colour with iodine (erythro-dextrin). Oxalic acid was found to be very much slower in its action than sulphuric acid.

O'Sullivan concluded from these results that all the eight preparations, if they could have been obtained in an absolutely pure condition, would have had no reducing action on Fehling's solution.

Elementary analyses were made with both the dextrins (erythro- and achroo-) after they had been perfectly freed from moisture in a current of dry air at 100° C. (212° F.); the results of which proved in a fairly conclusive manner that the composition of both was $C_6H_{10}O_5$.

It was found, on further treating any one of these dextrins with fresh malt-extract, that its reducing power (*K*) gradually increased to 66, and its rotatory power fell to $[\alpha]_D + 150^\circ$. When this stage had been arrived at all further action ceased. This remarkable stoppage of the action when a reducing power of *K* 66 had been reached was quite inexplicable, and led O'Sullivan to study the characters of the reducing body. The serious discrepancy between the results of experiments *a*, *b*, *c*, with those of *d*, in which, though the reducing powers of all the four were practically the same, there was a difference of some 6° or 7° in the rotatory power of *d*, pointed to the presence of some reducing body with a much higher power than glucose. This body proved to be maltose.

In 1875 Bondonneau,¹ presumably in ignorance of O'Sullivan's observations, described three dextrins α , β , and γ ; but as he was still under the impression that the sugar formed in the reaction was glucose, it is extremely probable, as pointed out by O'Sullivan, that the dextrin γ was an imaginary body, and that its supposed presence could be accounted for by taking into account the correct reducing power of maltose.

Discovery and Rediscovery of Maltose.—De Saussure in 1819² isolated a hitherto unknown sugar from the hydration products of starch, and described its crystalline nature. In 1847 Dubrunfaut³ further examined this new sugar and named it "maltose." He found it had an optical rotatory power three times as great as that of glucose, and that it was the sugar first formed when starch was acted upon by dilute acids. He described it as a crystalline body much less soluble in alcohol than glucose. Strange to say, these facts seem to have lapsed into comparative oblivion until C. O'Sullivan rediscovered and fully satisfied himself as to the existence of this sugar in 1872.⁴ He prepared it in the following manner, retaining the name "Maltose" given it by Dubrunfaut:

A conversion was made with 100 grammes of malt-starch at 40° C. (104°

¹ *Compt. rend.*, **81**, 972 and 1210.

³ *Ibid.* (3) **21**, 178.

² *Ann. Chim. Phys.*, **11**, 379.

⁴ *Journ. Chem. Soc.*, 1872, **25**, 579.

F.), the action being allowed to proceed for three hours. The solution was then boiled and filtered, the filtrate evaporated at a temperature of 80° C. (176° F.) to 300 c.c. The syrup thus obtained was boiled with two litres of alcohol of a specific gravity of 0.83, and allowed to cool. The clear liquid was then poured off from a syrup which was precipitated, and put on one side in a corked flask. At the end of six days the sides of the flask were covered with a crystalline crust, a portion of which, after being dried at 100° C. (212° F.) and dissolved in water, was found to possess an optical rotatory power of $[\alpha]_D^{25} + 150^\circ$ and a reducing power of (K) 65. He further found that the sugar was diffusible, and all attempts to further resolve it into other bodies by dialysis failed.

Further investigation by E. Schultze¹ confirmed the correctness of O'Sullivan's observations with reference to maltose.

C. O'Sullivan's Further Investigations.—In 1876 O'Sullivan² published an account of a further series of investigations on the action of malt-extract on starch. He concluded from a number of experiments, of which the following is an example, that maltose and dextrin are the only products of the action of malt-extract on starch :

One hundred grammes of potato-starch were intimately mixed with 200 c.c. of water at 55° to 60° C. (131° to 140° F.), and then 400–500 c.c. of boiling-water added with constant stirring. In this way a thorough gelatinisation of the starch was secured. The mixture was then cooled to 60° C. (140° F.) and the cold water extract³ from 10 grammes of pale malt added. In five or six minutes the paste dissolved, and the resulting solution was rapidly cooled and filtered. It was now examined, and found to have an optical rotatory power of $[\alpha]_D^{25} + 170.8^\circ$ and a reducing power of K 44, which figures are nearly equivalent to those of a mixture of 67.7 per cent. maltose and 32.3 per cent. dextrin. After being slowly evaporated the solid matter in solution had an optical rotatory power of $[\alpha]_D^{25} + 169.1^\circ$ and a reducing power of K 45.81, which figures are nearly equivalent to those of a mixture of 70.5 per cent. maltose and 29.5 per cent. dextrin.

A comparison of the figures actually obtained with those found by calculation for such a mixture show a very close agreement :

Observed.		Calculated.
170.8°		170.6°
169.1°		168.9

This tends to the assumption that maltose and dextrin alone are produced.

The solution was then evaporated to a thick syrup, placed, while still hot, over sulphuric acid in the bell-jar of an air-pump, and the air slowly exhausted. In a few hours a white, brittle, highly hygroscopic, porous mass was obtained. This was powdered and boiled with twenty times its bulk of alcohol of specific gravity 0.83. On cooling, the liquid separated into two portions, A, a thick syrup which fell to the bottom, and B, a clear supernatant solution. This latter

¹ *Ber. d. Deutsch. chem. Ges.*, **7**, 1047.

² *Journ. Chem. Soc.*, 1876, **29**, 125.

³ Made by mixing finely ground pale malt with water, allowing the mixture to stand for some hours at the ordinary temperature, and then filtering off the clear solution.

(B) was poured off and submitted to distillation. The resulting concentrated solution, after standing for a few days, crystallised to a solid mass, which was further purified by repeated re-crystallisation from alcohol. After being dried in a vacuum and then at 100°C . (212°F .), the crystalline substance was found to have an optical rotatory power of $[\alpha]_{\text{D}}^{25} + 148^{\circ}$, and a reducing power of K_{D}^{25} 66 to 67. When submitted to fermentation by yeast it was found to be entirely fermentable, yielding 51.5 per cent. of alcohol. The substance was evidently, therefore, maltose contaminated with malt-extract bodies.

The residual syrup had an optical rotatory power of $[\alpha]_{\text{D}} + 185.5^{\circ}$ and a reducing power of K 26.66, figures which approximate to those for a solution of 41 per cent. maltose and 59 per cent. dextrin. The optical rotatory power calculated for such a mixture is $[\alpha]_{\text{D}}^{25} + 187.76^{\circ}$, or 2.26° higher than that found; this discrepancy was attributed to the solubility of a portion of the malt-extract in alcohol.

The syrup A was evaporated in a vacuum to a solid state, and treated with alcohol in a similar manner to B; it separated into a syrup and a solution. The clear alcoholic solution yielded maltose. The solid matter of the precipitated syrup was found to have an optical rotatory power of $[\alpha]_{\text{D}}^{25} + 197.5$ and a reducing power of K 15.99, figures equivalent to 24.6 per cent. maltose and 75.4 per cent. dextrin. The optical rotatory power calculated for such a mixture is $[\alpha]_{\text{D}}^{25} + 198.2^{\circ}$.

The same operation was repeated several times with the syrup; the portions taken up by the alcohol were found in every case to be maltose, whilst the resultant syrups appeared to be mixtures of maltose and dextrin, the latter constituent gradually increasing on each successive treatment. When the stage had been reached which showed a reducing power equal to 10 or 12 per cent. maltose, it was found almost impossible to carry the separation further; but the optical rotatory powers and cupric reducing powers in all cases invariably pointed to the mixture consisting of maltose and dextrin only. This may be seen from the accompanying results, selected from a numerous series:

Rotatory Power Observed.	K_{D}^{25}	Maltose.	Calculated Rotatory Power.
$[\alpha]_{\text{D}}^{25} + 204.6^{\circ}$	9	13.8	205.17°
$[\alpha]_{\text{D}}^{25} + 205.5^{\circ}$	8	12.3	206.13°
$[\alpha]_{\text{D}}^{25} + 203.7^{\circ}$	10	15.3	204.21°

O'Sullivan concluded from the results of these experiments, and from those of numerous others performed under a great many varying conditions, that as all that portion which was soluble in alcohol was maltose, and since the comparison of the numbers actually found with those obtained by calculation invariably showed that, besides maltose, dextrin is the only other body present in the portion insoluble in alcohol, these two bodies must be, therefore, the sole products of the action of diastase on starch.

Cold Malt-Extract is without Action on Ungelatinised Starch.¹—

When a known weight of potato-starch was mixed with cold malt-extract, and

¹ Subsequent work has proved that starch granules are attacked at ordinary temperatures by malt-extract; some, however, to a greater extent than others.

at the end of twenty-four hours the liquid filtered off, it was found unaltered in specific gravity. Had there been any action, maltose and dextrin would have passed into solution, and these would have raised the specific gravity of the liquid. The starch, after being filtered off, washed, and dried in a vacuum for thirty-six to forty hours, was also found not to have lost weight. When, in a similar experiment, water-free starch was employed, a considerable rise of temperature was observed on mixing the starch and malt-extract, and the latter was found to have increased slightly in specific gravity. The starch, however, after being dried had not lost weight. When dehydrated starch was mixed with cold water, a similar rise in temperature took place, and this must be attributed to the chemical combination of water with the starch; hence the slight increase in gravity of the malt-extract was due to the loss of the small quantity of water which had entered into chemical combination with the starch.

Starch is Dissolved by Malt-Extract at the Temperature at which it Gelatinises, or a few Degrees Lower.—It was found that on adding malt-extract to starch and gradually raising the temperature, a portion dissolved at one point, a further portion at a higher stage, and so on. Complete solution was effected with potato-starch at 62° to 64° C. (144° to 147° F.). The small-grained starches, such as rice, maize, &c., were found to require a much higher temperature for their perfect solution; and in some cases the transforming power of the malt-extract was lost before the whole of the starch granules had been altered; but in these latter cases there was seldom more than 4 per cent. of the starch left undissolved.

Malt-Extract Dissolves Gelatinised Starch almost Completely in the Cold [10° to 20° C. (50° to 68° F.).]—15 c.c. of malt-extract were added to 5 grammes of gelatinised starch-paste cooled down to 16° C. (59° F.). After standing a few hours the solution was filtered from an insoluble portion (which did not amount to more than 4 per cent.). The filtrate gave no colour with iodine. After correction for the malt-extract the optical rotatory power was found to be $[\alpha]_{3.85} + 171^\circ$ and the reducing power $K_{3.85} 43.4$. These figures correspond with those of a mixture of 66.7 per cent. maltose and 33.3 per cent. dextrin. The calculated rotatory power for such a mixture is $[\alpha]_{4.88} + 171.3^\circ$, thus the agreement is very close. These are the constants which O'Sullivan usually found when the solution was filtered and analysed a few hours after the malt-extract had been added. When the action was allowed to continue for a greater length of time, the reducing power increased and the optical rotatory power diminished, especially when the malt-extract was present in large quantity. In another case, where 4 grammes of starch were treated with 15 c.c. malt-extract, and the action allowed to proceed for twenty-four hours, the constants found were equivalent to those of a mixture of 82 per cent. maltose and 18 per cent. of dextrin; in some cases constants equivalent to as much as 90 per cent. of maltose were obtained, in others to quantities varying between this and 67 per cent. The stronger the malt-extract employed and the more prolonged the digestion, the higher was the

reducing power and the lower the optical rotatory power of the transformation.

Influence of Temperature on the Rotatory Power and Cupric Reducing Power of Starch Conversions effected by Malt-Extract.—O'Sullivan was the first to study in a definite manner the influence of temperature, time, and concentration on these constants in the process of starch conversion by malt-extract.

In making conversions at varying temperatures, care was taken that the malt-extract was not used in excess, that is, in a quantity not more than was sufficient to effect the transformation in from five to ten minutes. He found that the action, which was at first extremely rapid, came to a sudden halt, and when this point had been reached, further action was exceedingly slow. It was found that the temperature at which the action was allowed to proceed decided to a great extent the point at which this halt took place; and by studying conversions conducted at different temperatures, O'Sullivan came to the conclusion that the action proceeded in three distinct phases, to each of which he assigned an equation. These he named the equations A, B, and C.

(A.) When a starch transformation, effected by the agency of malt-extract is conducted at any temperature below 63° C. (145° F.) and the solution filtered off in five or ten minutes, its optical rotatory power and reducing power always approximate closely to $[\alpha]_{3.85} + 170.6^\circ$ and $K_{3.85} 44.1$ respectively. These numbers correspond nearly with those of a mixture of 67.85 per cent. maltose and 32.15 per cent. dextrin. In the series of experiments which led to this conclusion, and of which a large number were undertaken, the malt-extract was heated to the temperature at which the conversion was to be made, then added to the starch-paste, and the whole maintained at that particular temperature until the termination of the transformation. The filtered solution gave no colour with iodine. The following, taken from a large number of similar experiments, are given as examples. In (a.) the transformation was effected at 30° C. (86° F.); (b.) at 40° C. (104° F.); (c.) at 50° C. (122° F.); and (d.) at 60° C. (140° F.).

	Apparent Percentage of Maltose.	Optical Rotatory Power Observed.	Optical Rotatory Power Calculated.
a.)	68.1	$[\alpha]_{3.85} = +169.7^\circ$	$[\alpha]_{3.85} = +170.4^\circ$
b.)	67.4	$[\alpha]_{3.85} = +170.7^\circ$	$[\alpha]_{3.85} = +170.8^\circ$
c.)	66.7	$[\alpha]_{3.85} = +169.9^\circ$	$[\alpha]_{3.85} = +171.4^\circ$
d.)	68.3	$[\alpha]_{3.85} = +170.8^\circ$	$[\alpha]_{3.85} = +170.2^\circ$

O'Sullivan concluded from this that the reaction under those conditions takes place according to a definite equation, which he designates A., thus:

and that the product consisted of 67.85 per cent. maltose and 32.15 per cent. dextrin.

When the malt-extract is not in excess, the solution can be kept for four

or five hours at the temperature at which the transformation is effected without showing any great change in its optical rotatory power and cupric reducing power. In such an experiment, where the solid matter of the malt-extract was equal to 4 per cent. of the weight of the starch taken, the transformation being effected at 55°C. (131°F.), and the solution kept at that temperature for five hours, the optical rotatory power was $[\alpha]_{\text{D}}^{25} + 169.5^{\circ}$ and the reducing power K 44.9. These numbers are nearly the same as those which would be given by a mixture of 68 per cent. maltose and 32 per cent. dextrin. Similar results were obtained in an experiment where the digestion had been carried on for eight hours.

When, however, malt-extract was used in excess, or when it contained more than the usual amount of acid, the maltose on prolonged digestion appeared to increase very slowly at the expense of the dextrin, whilst a small portion of the maltose itself was found to be hydrolysed to glucose.

(B.) When starch is dissolved by malt-extract at any temperature between 64° and 68° to 70°C. (147° and 155° to 158°F.), if the solution be immediately cooled and filtered, the product invariably gave constants equivalent to mixtures of 34.54 per cent. maltose and 65.46 per cent. dextrin; the optical rotatory power of such transformations being about $[\alpha]_{\text{D}}^{25} + 192.90$ and the reducing power K 22.4.

The following experiment is given as an example of numerous others, conducted with varying quantities of starch, and with starch from different sources (barley-malt, rice, maize, &c.), in which the apparent percentages of maltose produced never varied more than 2 per cent.

Malt-extract was heated to 66°C. (151°F.), and added to starch-paste at the same temperature. In four or five minutes the solution was cooled and filtered from a small amount of insoluble matter (0.5 per cent.). The solution gave no colour with iodine; its optical rotatory power was found to be $[\alpha]_{\text{D}}^{25} + 192.9^{\circ}$ and its reducing power K 22.4, numbers corresponding to those of a mixture of 34.5 maltose and 65.5 dextrin.

O'Sullivan considered that the transformation is expressed by the following equation, which he designates B.:

When the malt-extract was used in excess, or when it was more acid than usual, and the time of digestion was prolonged to several hours, an increase in reducing power and a decrease in optical rotatory power was observed similar to that in the case just described.

(C.) When starch is dissolved by malt-extract at temperatures from 68° to 70°C. (155° to 156°F.) to the point at which the activity of the diastase is destroyed, and the solution is cooled and filtered in five or ten minutes, the products have an optical rotatory power of $[\alpha]_{\text{D}}^{25} + 202.8^{\circ}$ and a cupric reducing power of K 11.3, agreeing closely with those for 17.4 per cent. of maltose and 82.6 per cent. of dextrin.

The following equation (C.) exhibits the change which is supposed to take place :

In numerous transformations conducted at temperatures between 69° to 76° C. (157° to 167° F.), the maltose apparently produced did not differ by more than 2 per cent.

The dextrin produced in this transformation can also be further degraded by employing excess of malt-extract, and by prolonging the time of digestion, as in the former cases A. and B.

O'Sullivan considers that the average quantity of malt-extract necessary to effect a starch conversion should contain solid matter equal to 5 per cent. of the starch taken, although, as he points out, malts differ very much in their starch-converting power.

It is suggested that the cause of this difference in the action of malt-extract on starch at varying temperatures is due most probably to the action of heat on the malt-extract. This may contain three transforming bodies—one which acts upon starch according to the A. equation, and has its activity destroyed at a temperature of 64° C. (148° F.); another which affects starch according to the B. equation, the action of which is arrested when a temperature of 68° to 70° C. (155° to 158° F.) is reached; whilst that which acts in accordance with equation C. is able to survive these temperatures.

In support of this it was found that if a portion of malt-extract was heated to 67° C. (153° F.) for a few minutes, cooled down to 60° (140° F.), and added to gelatinised starch at the latter temperature, the transformation was in accordance with equation B. and not with A. Similarly, by heating a portion of malt-extract to 75° C. (168° F.), and effecting a conversion at 68° C. (155° F.), the products of the action were in accordance with the C. equation.

Experiments of Musculus and Gruber.—In 1878 these observers published a paper¹ in which they sought to show that the achroodextrin of Brücke, the β -dextrin of O'Sullivan, and the dextrin II. of Griessmayer were not individual substances, but consisted of three dextrins, which they designated in the following manner :

Achroodextrin I.		$[\alpha] = +210^\circ$		κ 12
Achroodextrin II.		$[\alpha] = +199^\circ$		κ 12
Achroodextrin III.		$[\alpha] = +190^\circ$		κ 12

When these dextrins were isolated and treated with fresh diastase they showed the following remarkable modifications :

Achroodextrin I.		$[\alpha] = +159^\circ$		κ 36
Achroodextrin II.		$[\alpha] = +168^\circ$		κ 20
Achroodextrin III.		Unchanged.		

¹ *Bull. Soc. Chim.*, 30, 54.

They eventually considered the No. II. dextrin to be a mixture of Nos. I. and III., and in the latter portion of their paper only spoke of Nos. I. and III., which they called α and β . They also described another dextrin, which they styled γ . This was produced by adding fresh diastase to a starch conversion made at 50° or 60° C. (122° or 140° F.), and allowing the action to proceed for some time in the cold. It possessed an optical rotatory power of $[\alpha]_D + 150^{\circ}$, and a reducing power of $\kappa 28$. Though these bodies were obtained in an impure condition, still the remarkable difference in their behaviour when further treated with diastase pointed to striking differences in their respective characters. In these experiments malt diastase was employed.

It was in this paper that Musculus and Gruber first enunciated the hypothesis that when starch breaks down under the agency of diastase, the action proceeds in successive stages; the starch molecule splitting up first into maltose and a dextrin having a molecular weight nearly as high as that of starch. This newly-formed dextrin then further splits up into maltose and a dextrin of less molecular weight, and so on, until the last dextrin is produced, which is simply hydrolysed to maltose. They were led by these considerations to regard starch as a polysaccharide containing the group $C_{12}H_{20}O_{10}$ five or six times.

Investigations of H. T. Brown and Heron.—The hypothesis of Musculus and Gruber received further confirmation in the results of a series of experiments undertaken by Brown and Heron which were communicated to the Chemical Society in 1879.¹ These authors employed potato-starch which, after being purified by successive treatment with dilute solution of caustic soda and 1 per cent. hydrochloric acid, was finally washed until free from all traces of acid. Great care was taken to insure thorough gelatinisation of the starch used in the experiments. It was evenly mixed with cold water, and then poured into the necessary quantity of boiling water with constant stirring. After the gelatinised starch paste had been cooled down to the temperature at which the transformation was to be effected, the necessary quantity of malt-extract was dropped in either from a burette or a pipette, and the whole was then placed in a water-bath maintained at the required temperature by means of a thermo-regulator. Since the authors were not satisfied with the method then in use for preparing diastase, malt-extract was invariably employed. This was prepared by intimately mixing together 100 grammes of finely ground pale malt with 250 c.c. of distilled water, the mixture being allowed to stand for from six to twelve hours. The bright filtrate from this, which was designated normal malt-extract, and which had a specific gravity of from 1036 to 1040 (water = 1000), was used in the majority of the experiments. The specific gravity, optical rotatory power, and cupric reducing power were determined either before or after heating, according to the nature of the experiment. At the end of each conversion the quantity of liquid was either measured or weighed, and its volume calculated from its specific gravity. All portions removed for the purpose of testing with iodine were carefully measured

¹ *Journ. Chem. Soc., Trans.*, 1879, **35**, 596.

and allowed for. In those cases where more than 3 c.c. of malt-extract were used, or in which the transformation lasted more than twenty minutes, a portion of malt-extract was digested alongside the conversion in a separate vessel, and in this way submitted to the same conditions of time and temperature as the starch conversion itself. This precaution was found necessary, since malt-extract was found to change rapidly even at ordinary temperatures; its specific gravity and cupric reducing power increasing, and its optical rotatory power diminishing. Malt-extract rapidly enters into a state of active fermentation, caused by the presence of various bacterial organisms. If kept at a temperature of 40° to 45° C. (104° to 113° F.), bacteria alone appear, and a fermentation accompanied with the copious evolution of carbon dioxide and hydrogen gases makes its appearance, the chief product of the fermentation being butyric acid. But the changes in the optical rotatory power and chemical conditions above mentioned do not depend upon the presence of organised ferments, for the same changes take place when the solution has been heated to a temperature high enough to paralyse such organisms. Malt-extract, after having been heated to a temperature of 75° C. (165° F.), may be kept at a temperature of 50° C. (122° F.) without undergoing much change, and this shows that the alteration is not due to hydrolysis caused by the acids present in the malt-extract. The change was partly attributed to the action of an enzyme always present in malt, which possesses the power of hydrolysing the cane-sugar invariably present in the malt-extract; partly also to the action of the diastase on some of the starch granules which are unavoidably ruptured during the process of grinding the malt. The contents of these suffer hydrolysis and are partially converted into maltose.

Action of Diastase on Unruptured Starch.—The previous observations of O'Sullivan (p. 140) were confirmed, it being found that diastase was entirely without action on unruptured potato-starch.

Action of Diastase on Bruised Starch.—When starch was ground up in a mortar with quartz sand or powdered glass, and then exposed to the action of malt-extract, it was readily affected by diastase in the cold. The following are the results of an experiment conducted in this way, the bruised starch being acted upon by a solution containing 10 c.c. of normal malt-extract in every 100 c.c.:

Matter found in solution = 3.155 grammes.

	[α] _{3.86} .	[κ] _{3.86} .	Factors corresponding to Mixtures of		
			Maltose.	Dextrin.	Cellulose.
			Per Cent.	Per Cent.	Per Cent.
After 4 hours	+155.4°	51.0	83.6	13.9	2.5
After 20 hours	+152.0°	54.4	89.1	8.5	2.4

All the transformations had very low optical rotatory power, and when these were compared with the reducing power, the two did not coincide with the assumption that maltose and dextrin only were present. This discrepancy was attributed to the presence of a small quantity of starch cellulose which had passed into solution.¹

Action of Malt-Extract upon Gelatinised Starch in the Cold.—This was found to be extremely rapid, for on treating a 3 to 4 per cent. solution of gelatinised starch with 5 to 10 c.c. of normal malt-extract, limpidity ensued in from one to three minutes; immediately afterwards iodine ceased to give a blue colour; the brown colour, however, persisted for another five or six minutes. When this point was reached, the solution could be filtered off quite bright and clear, and its optical rotatory power was found to be at its lowest point. This and the reducing power do not point to the apparent exclusive presence of maltose and dextrin; evidently a little cellulose also goes into solution. (See, however, footnote to previous paragraph.) After a time a deposition of starch cellulose takes place, rendering the liquid turbid. Concurrently with this the optical rotatory power and cupric reducing power slowly begin to rise, arriving at their maxima in about three hours, when they now coincide with the apparent presence of maltose and dextrin alone in the solution. The following is a typical experiment :

3.7 Grammes of Starch, 10 c.c. Normal Malt-Extract in 100 c.c.

Time.	$[\alpha]_{3-86}$.	κ_{3-86}	Iodine Reaction.
5 minutes	154.6°	...	Slightly brown
15 minutes	145.6°	...	None
30 minutes	154.6°	...	None
60 minutes	157.5°	44.1	None
3 hours	161.6°	49.7	None

Action of Malt-Extract, which had been Previously Heated, upon Gelatinised Starch in the Cold.—In this case, though the power of converting starch into soluble starch, as shown by the solution rapidly liquefying, did not suffer much alteration, the saccharifying action, as shown by the iodine test and by the optical rotatory power of the liquid, was rendered much slower, and this in proportion as the temperature to which the malt-extract had been previously subjected was increased. Transformations made with malt-extract which had been previously heated to 66° C. (151° F.) were much more transparent than those in which unheated malt-extract was employed.

Action of Malt-Extract upon Gelatinised Starch at Elevated Temperatures.—In order to be able to study the changes taking place at any given

¹ Brown and Morris (*Journ. Chem. Soc., Trans.*, 1895, **67**, 309) have observed that this discrepancy is not due to the cause here stated, but to the fact that the optical rotatory power of maltose, when first produced by hydrolysis, is somewhat lower than when its solution has been boiled or has stood for a time,

point in a starch transformation, it was requisite to discover some method by means of which diastatic action could be arrested at any given point. Boiling the solution, though it completely arrested the further action of diastase, was unsatisfactory; changes invariably took place during the time required to bring the liquid to the boiling-point. Brown and Heron found that salicylic acid fulfilled this condition, for when it was added to transformations in quantities of 0.05 gramme to each 100 c.c. of the solution, all further diastatic action was completely arrested.¹ It became now a comparatively easy matter to show by means of a curve the progress of any starch transformation. The authors agree with the previous observations of O'Sullivan (p. 142) that the changes brought about in the malt-extract by the agency of heat are the cause of the differences in its behaviour as a transforming agent, but they were unable to obtain the various halting stages described by O'Sullivan when the processes were carried out at the respective temperatures indicated by him.

Action of Malt-Extract in Transformations conducted at 40° C. (104° F.).—When a conversion was made at this temperature, the process differed in several respects from one conducted in the cold. Unless the starch-paste was very thick, or the malt-extract very small in quantity, no separation of starch cellulose took place,² for, instead of being precipitated it was converted into maltose and dextrin. The solution consequently remained clear, and its optical rotatory power and cupric reducing power coincided with the apparent presence of maltose and dextrin only. The transformation proceeded with great rapidity, and the halting stage, which seemed to be exactly at the same point as when the process took place in the cold, was reached in about thirty minutes. When this stage was attained, further action was exceedingly slow, even when further quantities of the same (*i.e.*, the heated) malt-extract were added. The following are the details of an experiment in which a solution of 5 grammes of starch per 100 c.c. of water were acted upon by normal malt-extract which had been heated for twenty minutes to 40° C. (104° F.), 10 c.c. malt-extract being added for each 100 c.c. of solution :

Temperature of the Conversion 40° C. (104° F.).

Time.	$[\alpha]_{D-86}^{\circ}$	n_{D-86}°	Iodine Reaction.
2½ minutes	+ 164.1°	...	Full brown
15 minutes	+ 163.3°	...	Brown
30 minutes	+ 163.3°	48.8	None

These last figures correspond closely with those of a mixture of 80 per cent. maltose and 20 per cent. dextrin.

¹ It is now known that salicylic acid of this concentration does not arrest diastatic action.

² See footnote, p. 147.

After a further addition of 10 c.c. of the same malt-extract per 100 c.c. of solution, the process was allowed to proceed for thirty minutes longer; the optical rotatory power was $[\alpha]_{j3^{\circ}86} + 161.6^{\circ}$ and the reducing power $\kappa_{3^{\circ}86}$ 49.7, numbers corresponding with those of a mixture of 81.3 per cent. maltose and 18.7 dextrin. Fermentation had now set in, and this precluded further observation.

Action of Malt-Extract at 50° C. (122° F.).—In a transformation conducted at 50° C. with malt-extract, which had been previously heated to that temperature for twenty minutes, and added at the rate of 10 c.c. to each 100 c.c. of 5 per cent. starch solution, the following results were obtained :

Time.	$[\alpha]_{j3^{\circ}86}$	$\kappa_{3^{\circ}86}$	Iodine Reaction.
2½ minutes	+ 168.1°	...	Full brown
5 minutes	+ 166.5°	...	Brown
15 minutes	+ 166.5°	...	Brown
30 minutes	+ 162.7°	...	No coloration
60 minutes	+ 162.3°	49.6	No coloration

The last figures correspond with those of a mixture of maltose 81.3 per cent. and dextrin 18.7 per cent.

On the addition of a further quantity of malt-extract there was at first little or no action, but after standing for sixteen hours the optical rotatory power had fallen to $[\alpha]_{j3^{\circ}86} + 147.7^{\circ}$ and the reducing power had risen to $\kappa_{3^{\circ}86}$ 60.8, numbers which correspond closely with the constants of maltose.

Action of Malt-Extract Heated to 60° C. (140° F.).—The following are the results of an experiment similar to the preceding, in which the transformation was conducted at 60° C. with malt-extract which had been previously heated for twenty minutes to that temperature, the further action of the diastase being arrested by salicylic acid at the various points indicated :—

Time.	$[\alpha]_{j3^{\circ}86}$	$\kappa_{3^{\circ}86}$	Iodine Reaction.
1 minute	+ 191.7°	...	Pure blue
2½ minutes	+ 175.6°	...	Brown
5 minutes	+ 166.8°	...	Very light brown
15 minutes	+ 165.7°	..	None
30 minutes	+ 163.7°	...	None
60 minutes	+ 162.9°	49.6	None
More malt-extract added at the rate of 4.5 c.c. per 100 c.c.			
90 minutes	+ 161.3°	...	...
More malt-extract added at the rate of 4.2 c.c. per 100 c.c.			
120 minutes	+ 160.1°	52.0	...

An optical rotatory power of $[\alpha]_{j3^{\circ}86} + 162.6^{\circ}$ and a reducing power of $\kappa_{3^{\circ}86}$ 49.3 was deduced as the mean of a large number of starch transformations conducted at a temperature of 60° C. (140° F.). In these from 19 to 20 c.c. of malt-extract per 100 c.c. of starch solution were used, and the conversions

allowed to proceed for lengths of time varying from five to sixty minutes. These numbers correspond very closely with those of a mixture of 81.3 per cent. maltose and 18.7 dextrin.

This was the temperature at which O'Sullivan states that he obtained his A. equation, the halting stage being reached when the conversion had an optical rotatory power of $[\alpha]_{j_{3.85}} + 170^\circ$ and a reducing power of $K_{3.85}$ 44.1. Brown and Heron were, however, unable to detect any pause in the reaction at this point.

Action of Malt-Extract Heated to 66° C. (151° F.).—If malt-extract be heated rapidly to 66° C. and then immediately added to a solution of gelatinised starch maintained at that temperature, the reaction takes place in its earlier stages very much in accordance with that of a transformation conducted with malt-extract heated to 60° C. (140° F.); but if the malt-extract be kept for ten or fifteen minutes or longer at 66° C., its action is considerably modified. Its general character is much slower, although the formation of soluble starch, as indicated by complete limpidity in the liquid, is accelerated. The following is an illustration of such a conversion in which malt-extract, previously heated to 66° C. for twenty minutes, was added in the proportion of 8.92 c.c. to every 100 c.c. of a 6 per cent. starch solution. The temperature of the conversion was 65° C., and the action was arrested by salicylic acid at the points indicated :

Time.	$[\alpha]_{j_{3.86}}$	Iodine Reaction.
2½ minutes	+ 204.0°	Full brown
5 minutes	+ 201.2°	Brown
10 minutes	+ 195.5°	Light brown
20 minutes	+ 191.8°	Very light brown
30 minutes	+ 188.2°	No reaction
60 minutes	+ 185.0°	No reaction
Same malt-extract added at the rate of 4.3 c.c. per 100 c.c.		
90 minutes	+ 178.0°	...
Same malt-extract added at the rate of 4.3 c.c. per 100 c.c.		
120 minutes	+ 178.0°	...

The point at which the iodine reaction for erythrodextrin disappeared was markedly different in transformations conducted at 66° C. and in those at 90° C. In the former the disappearance of the brown colour took place when the optical rotatory power had fallen to $[\alpha]_{j_{3.86}}$ 188° or 189°, whilst in the latter it was still to be observed when the angle had fallen as low as $[\alpha]_{j_{3.86}}$ 165° or 166°. The reason assigned for this was, that during the process of heating the various transforming agents in the malt-extract suffer alteration in different degrees; thus in transformations conducted at 60° C. (140° F.) those portions of the malt-extract whose function it is to split up and degrade the higher achroodextrins effect this more rapidly than the erythrodextrins can be produced or transformed into the higher achroodextrins; consequently, much maltose and much of the lower achroodextrins may be present in the

solution before the erythrodextrins and soluble starch are converted into achroodextrins.

Action of Malt-Extract at Temperatures Higher than 66° C. (151° F.).—When malt-extract is heated to a temperature between 66° C. and 75° to 76° C. (167° to 169° F.) its action becomes further modified. A lower optical rotatory power than $[\alpha]_{\text{D}}^{20} 195^\circ$ is seldom reached, even on the addition of further quantities of the same malt-extract and prolonged digestion.

The following are the results of an experiment conducted at 76° C. with 30 c.c. of malt-extract heated to 76° C. for each 100 c.c. of starch solution :

Time.	$[\alpha]_{\text{D}}^{20}$	κ_{D}^{20}	Iodine Reaction.
2½ minutes	+205.0°	...	Much erythrodextrin
5 minutes	+200.3°	...	Much erythrodextrin
10 minutes	+196.5°	...	Slight coloration
20 minutes	+195.5°	...	No reaction
30 minutes	+195.5°	...	No reaction
45 minutes	+194.6°	...	No reaction
60 minutes	+194.6°	23.0	No reaction
15 c.c. same malt-extract added per 100 c.c.			
70 minutes	+194.4°	...	...
90 minutes	+194.0°	...	...

The starch solution became limpid very quickly, and the blue or violet reaction with iodine, indicative of starch, disappeared in less than two minutes, but the brown colour due to erythrodextrin persisted for a considerable time. This latter attained its highest point when the optical rotatory power had reached $[\alpha]_{\text{D}}^{20} + 202^\circ$ to 203° , and disappeared at 194° or a little lower.

This corresponds very closely with O'Sullivan's C. equation, which has an optical rotatory power of $[\alpha]_{\text{D}}^{20} + 202.8^\circ$, but the author describes the dextrin obtained from it to be an achroodextrin. Brown and Heron invariably found it was at this point that the solution gave the strongest brownish-red colour with iodine, characteristic of erythrodextrin. They concluded that in these cases the dextrin formed was invariably an erythrodextrin.

Influence of Neutralisation upon the Action of Malt-Extract.—When malt-extract, after being heated to 66° C. (151° F.), was neutralised with barium hydroxide, its action was but little modified, and the curve given by a transformation with malt-extract treated in this way did not differ materially from one made with malt-extract which had been simply heated to 66° C.

If, however, the malt-extract be heated to 66° C., rendered very slightly alkaline with sodium carbonate, and again momentarily heated to 66° C., the optical rotatory power of the solution was reduced rapidly to $[\alpha]_{\text{D}}^{20} + 194^\circ$ to 196° , and it was impossible to push the action beyond this, even by the addition of further quantities of similarly treated malt-extract. The curve obtained in this way did not differ materially from that obtained with malt-extract simply heated to 75° to 76° C. (167° to 169° F.).

When a conversion was made with malt-extract heated to 66° C. (151° F.),

and then made slightly alkaline with sodium hydroxide, the optical rotatory power never fell below $[\alpha]_{j3^{\circ}86} + 202^{\circ}$. This point corresponds with that at which iodine gives the maximum reaction for erythrodextrin in conversions made with malt-extract heated to from 66° to 76° C. (141° to 169° F.). Further additions of the same alkaline malt-extract failed to bring down the angle farther.

When the malt-extract was rendered still more alkaline with sodium hydroxide, its diastatic power was completely destroyed.

The Molecular Transformation of Starch.—Brown and Heron consider that the results of these experiments show that there are at least four well-defined molecular transformations of starch, the one most easily obtained being that with malt-extract heated to a temperature not higher than 60° C. (140° F.). This has an optical rotatory power of $[\alpha]_{j3^{\circ}86} + 188.5^{\circ}$, and a reducing power of $\kappa_{3^{\circ}86} 49.3$.

The next is that at which the iodine reaction for erythrodextrin disappears in transformations conducted with malt-extract heated to 66° C. (151° F.). Its optical rotatory power is $[\alpha]_{j3^{\circ}86} = +188.5^{\circ}$, and its reducing power about $\kappa_{3^{\circ}86} 25.0$.

The next is obtained by using malt-extract heated to 66° C., and made slightly alkaline with sodium carbonate. Its optical rotatory power is $[\alpha]_{j3^{\circ}86} + 195^{\circ}$ to 196° , and its reducing power $\kappa_{3^{\circ}86} 18.9$.

The last is obtained by using malt-extract heated to 66° C., and made slightly alkaline with sodium hydrate; also in conversions conducted at temperatures beyond 66° C. (151° F.), when the iodine reaction for erythrodextrin is at its maximum. It has an optical rotatory power of $[\alpha]_{j3^{\circ}86} + 202^{\circ}$ to 203° , and a reducing power of $\kappa 12.7$. This agrees closely with O'Sullivan's C. equation; the dextrin formed is, however, an erythrodextrin.

Transformations from Higher to Lower Equations.—If a little unheated malt-extract be added to any starch transformation having an optical rotatory power higher than $[\alpha]_{j3^{\circ}86} + 162.5^{\circ}$, and the whole be kept at a temperature of 50° to 60° C. (122° to 140° F.), the rotation of the solution falls very quickly, in favourable cases almost instantaneously, to $[\alpha]_{j3^{\circ}86} = 162.5^{\circ}$, the reducing power rising to $\kappa_{3^{\circ}86} 48.3$, at which point it remains stationary for some time. For instance, 5 c.c. unheated malt-extract were added to 100 c.c. of a starch conversion which had been boiled, and which had a rotatory power of $[\alpha]_{j3^{\circ}86} 187.8^{\circ}$ and a reducing power of $\kappa_{3^{\circ}86} 28.9$; at the end of two minutes the former had fallen to 162.6° and the latter had risen to $\kappa_{3^{\circ}86} 49.3$.

The remarkable fact that hydrolysis proceeds rapidly to a certain point and then becomes suddenly arrested, points to a marked difference in character between the higher and the lowest dextrin. The most natural explanation is that the dextrans are not metameric but polymeric bodies, those which possess the highest optical rotatory power having the largest molecules.

In consonance with the view of Musculus and Gruber (p. 145), Brown and

Heron regard soluble starch as a polysaccharide. They represent it by the formula: $10C_{12}H_{20}O_{10}$. The first step in the breaking down of this large complex molecule is the removal of one of the $C_{12}H_{20}O_{10}$ groups, which, uniting with a molecule of water, becomes maltose; and the remaining nine $C_{12}H_{20}O_{10}$ groups constitute the first dextrin of the series—erythrodextrin α , thus—

This dextrin next suffers decomposition; one of its $C_{12}H_{20}O_{10}$ groups is similarly split off, combines with a molecule of water to form maltose, and the remaining eight $C_{12}H_{20}O_{10}$ groups constitute the second dextrin of the series—erythrodextrin β —

The next stage is:

The process goes on in a similar manner until the last $C_{12}H_{20}O_{10}$ group is transformed into maltose.

It is obvious that the number of dextrins possible will depend upon the size of the molecule of the last formed dextrin; if this contains the same number of carbon atoms as maltose, there will be probably nine distinct stages; if it contain twice as many, then the number can be only eight. The following table gives the optical rotatory power and reducing power in each of these theoretical stages, and the mixtures of maltose and dextrin with which these factors correspond:

No. of Equation.	Optical Rotatory Powers	Reducing Power.	Apparent		
			Maltose.	Dextrin.	
	$[\alpha]_{D^{20}}$	$\kappa_{D^{20}}$	Per Cent.	Per Cent.	
Soluble starch	+216.0°	0.0	...	...	...
1	+209.0°	6.4	10.5	89.5	Erythrodextrin α
2	+202.2°	12.7	20.9	79.1	Erythrodextrin β
3	+195.4°	18.9	31.0	69.0	Achroodextrin α
4	+188.7°	25.2	41.3	58.7	Achroodextrin β
5	+182.1°	31.3	51.3	48.7	Achroodextrin γ
6	+175.6°	37.3	61.1	38.9	Achroodextrin δ
7	+169.0°	43.3	71.0	29.0	Achroodextrin ϵ
8	+162.6°	49.3	80.9	19.1	Achroodextrin ζ
9	+156.3°	55.1	90.2	9.8	Achroodextrin η
Maltose	+150.0°	61.0	100.0	0.0	

Brown and Heron considered that they had unmistakably established by their experiments the existence of the No. 2, 3, 4, and 8 equations, whilst indications of the others had been observed, though not with the same degree

of certainty. Of these equations No. 8 was by far the most stable; and as no pause could be observed in the process of hydration between it and maltose, it favoured the view that there are only eight dextrins. The result of all these experiments tended to show that soluble starch, when hydrolysed by malt-extract, was invariably split up into maltose with an optical rotatory power of $[\alpha]_{j3^{\circ}86} + 150^{\circ}$ and a reducing power of $\kappa_{3^{\circ}86}$ 61, and a series of polymeric dextrins, all of which had an optical rotatory power of $[\alpha]_{j3^{\circ}86} + 216^{\circ}$ and possessed no reducing power.

Diastase has no Action on Maltose.—A number of experiments were made to ascertain if diastase possessed the power of hydrolysing maltose. These were attended with entirely negative results; in no case was glucose found even after the maltose had been subjected to the action of malt-extract for twenty-eight hours.

Investigations of H. T. Brown and Morris.—In 1885 Brown and Morris published a paper¹ which may be regarded as a continuation of that of Brown and Heron of 1879. In this they give the results of a number of experiments undertaken with a view of still further elucidating the molecular changes which take place when starch is hydrolysed.

It had been previously pointed out by Brown and Heron that when starch paste is acted on by malt-extract at any temperature above 40° C. (104° F.), the rotatory and reducing power of the products both appear to indicate the existence of maltose $[\alpha]_{j3^{\circ}86} + 150^{\circ}$, $\kappa_{3^{\circ}86}$ 61, and a non-reducing dextrin with a rotatory power of $[\alpha]_{j3^{\circ}86} + 216^{\circ}$. They now showed that the same held good with the various fractions obtained by precipitation with alcohol; the rotatory and reducing powers of such fractions invariably pointed to their being apparently composed of maltose and a non-reducing dextrin. A large number of experiments were made in proof of this, of which the following is an example:—A starch conversion was made which gave a rotatory power of $[\alpha]_{j3^{\circ}86} + 196.4^{\circ}$ and a reducing power of $\kappa_{3^{\circ}86}$ 18.6, figures which correspond closely to a composition of maltose 30.4 per cent. and dextrin 69.6 per cent. The conversion was evaporated to a syrup, heated to 100° C. (212° F.), and 90 per cent. of alcohol added until a permanent precipitate was produced. The solution, which was found to contain 48.3 per cent. of alcohol, was then allowed to become cold, and the clear supernatant liquid poured off from the residue. As this latter chiefly consisted of malt-extract bodies, it was neglected. The clear liquid was heated on the water-bath, more alcohol added until a permanent precipitate again formed, when it was cooled, the precipitate allowed to settle, and the clear liquid, which was found to contain 48.34 per cent. of alcohol, poured off. This same process was repeated five times, consequently five fractions and a mother-liquid were obtained. Each separate fraction was dissolved in water and re-precipitated with alcohol, again taken up with water, boiled to remove alcohol, evaporated to small bulk, and finally dried in a vacuum. The

¹ *Journ. Chem. Soc., Trans.*, 1885, 47, 527.

rotatory and reducing power of each fraction are given in the following table :

Fractions.	Strength of Alcohol.	Optical Rotatory Powers.	Reducing Powers.	Apparently indicating	
				Maltose.	Dextrin.
	Per Cent.	$[\alpha]_{D^{86}}$.	$\kappa_{D^{86}}$.	Per Cent.	Per Cent.
1	48.3	+209.8°	5.49	9.0	91.0
2	59.4	+209.6°	8.85	14.5	85.5
3	67.0	+203.1°	9.00	14.7	85.3
4	74.0	+202.4°	13.18	21.6	78.4
5	81.5	+199.1°	14.53	13.8	73.2
Mother-liquor . . .	...	+185.6°	29.40	48.1	51.9

The results of this and numerous experiments of a similar nature led Brown and Morris to the conclusion that "just as the total products of all starch transformations brought about by malt-extract always yield numbers indicating the existence of maltose and a non-reducing dextrin only, so it is equally beyond question that the same holds good with regard to any portion of those products which can be separated by fractionation with alcohol."

They claimed to have thus "established a criterion of purity for the products of the action of diastase on starch." And "in those cases in which the observations of other writers do not conform to the above rules," they "have no hesitation in ascribing the discrepancy either to the presence of impurities or to errors in analysis." In more recent papers they speak of this as the "law of definite relation"; consequently, if the optical rotatory power of a starch transformation is known, the reducing power can be deduced from it, and *vice versa*.

As had been previously shown (p. 149), starch transformations of a higher rotatory power than $[\alpha]_{D^{86}} 162.6^\circ$ and a lower reducing power than $\kappa 49.3$ are speedily brought down to this stage by being treated with a little fresh (unheated) malt-extract at 50° C. (122° F.). Since maltose is unaffected by this treatment, the change must be owing solely to a degradation of the dextrins.

After making sure that the dextrins suffered no change by the various boilings, evaporations, and precipitations with alcohol necessary to secure their isolation, experiments were made to ascertain if, when isolated, they could be degraded by treatment with fresh malt-extract, it could be conclusively shown that the dextrin when thus isolated could be degraded as readily as the original substance from which they were prepared. Each dextrin, then, with the exception of that belonging to the No. 8 equation, when treated with fresh malt-extract at 50° C. (122° F.) ought to yield a certain definite amount of maltose. The different quantities which should be yielded in this way are given in the accompanying table :

No. of Equation.	Constants of Mixed Products.		Apparent Yield of Maltose by 100 Parts of each Dextrin when Degraded to No. 8 Equation.
Soluble starch	$[\alpha]_{3-86}.$	$[\kappa]_{3-86}.$	
1	+ 216.0°	0.0	84.44
2	+ 209.0°	6.4	82.09
3	+ 202.2°	12.7	79.20
4	+ 195.4°	18.9	75.39
5	+ 188.7°	25.2	70.37
6	+ 182.1°	31.3	63.33
7	+ 175.6°	37.3	52.77
8	+ 169.0°	43.3	35.18
	+ 162.6°	49.3	0.00

On ascertaining the amount of maltose yielded by any one of the dextrans, it ought to be possible, by the aid of this table, to fix its position in the series, or with a mixture of dextrans to find their mean position. It was shown by a number of experiments, of which the following is an example, that all transformations which had higher optical rotatory powers and lower reducing powers than those required for the No. 8 equation, always contained several, and never one individual dextrin. A starch transformation was made with a little freshly prepared diastase, the action being arrested when the maximum of coloration was given by iodine. This possessed an optical rotatory power of $[\alpha]_{3-86} + 202.2^\circ$, and a reducing power of κ_{3-86} 12.4, which are almost exactly those of the Equation 2, and which closely corresponded to those of a mixture of maltose 20.3 per cent. and dextrin 79.7 per cent. On further degradation with malt-extract at 60° C. (140° F.), 75 per cent. of maltose was yielded, against 79.2 per cent. as required by theory.

A measured quantity of this transformation was then evaporated to a syrup and fractionated with alcohol of gradually increasing strength. The weight of each fraction was taken and calculated as a percentage on the whole, and the amount of maltose apparently yielded by each fraction on subsequent degradation with fresh malt-extract also. These were found to be as follows :

Fractions.		Strength of Alcohol.	Percentage of Solid Matter to Total Solid Matter.	Maltose apparently Yielded by each 100 Parts of Dextrin.
		Per Cent.		Parts.
1		46.0	43.88	60.8
2		59.3	23.09	70.8
3		75.0	10.64	87.7
4		...	22.39	93.9

The true mean of these figures gives 73.3 per cent. maltose against the 75 per cent. yielded by the original solution, numbers which agree as closely as could be expected on an experiment of this difficult nature. These experiments show that though it is possible to give theoretically well-marked equations

representing the hydrolysis of starch, yet in practice, with the exception of the No. 8 equation, no such well-defined equation is met with. The action is evidently exceedingly irregular, some of the $C_{19}H_{20}O_{10}$ groups being hydrolysed more quickly than others; consequently, the optical rotatory power and cupric reducing power of any transformation having a higher rotatory power than that of the No. 8 equation does not represent one definite equation, but the mean of several.

It was then suggested that the starch molecule might be even more complex than the authors had hitherto assumed it to be in their former paper, where it was considered to be equal to ten ($C_{12}H_{20}O_{10}$) groups, and that it might be some simple multiple of these ten groups. In such a case the number of dextrans would be correspondingly increased.

Brown and Morris now attempted to obtain the dextrans in a state of purity. The opinions of various observers have differed considerably as to whether the dextrans do or do not possess a reducing action on Fehling's solution. In the experiments of O'Sullivan previously described (p. 138), the reducing power they were found to possess was attributed to traces of maltose which it seemed impossible to completely remove. An unsuccessful attempt to destroy these last traces of maltose was made by Brown and Morris by boiling with Fehling's solution. Bondonneau¹ described a method by means of which he thought he had succeeded in accomplishing this object. It consisted in boiling the dextrin solution for half-an-hour with cupric chloride, to which just sufficient sodium hydrate had been added as would redissolve the precipitated cupric hydrate. The filtered liquid, on being acidulated with hydrochloric acid, was said to yield, on the addition of alcohol, a dextrin entirely free from maltose. Brown and Morris made several attempts to prepare a non-reducing dextrin by this method, but never succeeded in obtaining one with a lower reducing power than κ 0.53. Wiley in 1882² designed a method for the analysis of mixtures of maltose, glucose, and dextrin, in which Knapp's mercuric cyanide solution was employed in a novel manner. It was used to destroy the maltose and glucose, under the supposition that the dextrin would be left intact, and that its amount could be afterwards estimated by the polarimeter. By working with this solution, Brown and Morris were able to deprive several of the dextrans of every trace of reducing power, and this they attributed to the complete removal of maltose (*cf.*, however, Scheibler and Mittelmeier's views, p. 78; also Brown and Millar, p. 177). The following is an experiment of this kind:

A dextrin was prepared from a starch transformation and purified by precipitation and re-precipitation several times with 60 per cent. alcohol, the spirit employed being always of the same strength, in order to avoid any alteration in its composition. A slight excess of a solution of equal weights of mercuric cyanide and sodium hydroxide³ was added to a solution of this

¹ *Bull. Soc. Chim.*, 21, 50 and 159.

² *Chemical News*, 46, 175.

³ Morris (*Trans. Lab. Club*, i. 70) states that a preferable method is to treat the dextrin solution on the water-bath with mercuric oxide and barium hydrate solution.

dextrin, and the whole kept warm until reduction was deemed complete. The solution was cooled, acidified with hydrochloric acid, and hydrogen sulphide passed through until all the mercury was removed. Ammonia was now added in slight excess to the filtered solution, the whole evaporated to a syrup; this latter was dissolved in hot water, and the dextrin precipitated therefrom with 60 per cent. alcohol. In this way they believed that they had obtained a dextrin entirely devoid of reducing power. This, when degraded with malt-extract at 60° C. (140° F.), gave (after corrections had been made for the salts present) 51.7 per cent. maltose against 49.5 per cent. yielded by the original dextrin; thus it appeared to have suffered no alteration during the process of purification. Its optical rotatory power was $[\alpha]_{D^{33.86}} 215.9^\circ$, a value which agrees almost exactly with that which the authors had previously deduced from their experiments on the transformation of starch, *i.e.* $[\alpha]_{D^{33.86}} + 216.0^\circ$.

Maltodextrin.—The discrepancy in the results of the experiments where the dextrins were degraded with fresh malt-extract (an illustration of which is given on p. 156, where it is seen that while fractions 1 and 2 yield considerably less maltose, 3 and 4 yield considerably more than the quantity required by theory) is explained by the presence of a body which the authors had isolated and named “maltodextrin.” It was obtained in the following manner :

Preparation of Maltodextrin.—A starch transformation was made at a temperature of 60° to 65° C. (140° to 149° F.) with a little freshly prepared diastase, the action being arrested by boiling the solution when its optical rotatory power had reached $[\alpha]_{D^{33.86}} + 198^\circ$ or thereabouts. The liquid was then evaporated to a specific gravity of 1060, and set to ferment at 28° to 30° C. (82° to 86° F.) with a small quantity of ordinary yeast. When fermentation had ceased, the liquid was filtered, evaporated to a syrup, and digested in boiling 90 per cent. alcohol for a couple of days, with frequent agitation. The object of this treatment was to remove the non-volatile products of fermentation, which amounted to about 5 per cent. of the maltose fermented. The strength of the alcohol was then reduced to 85 per cent., the mixture submitted to further digestion, and the liquid portion, while still hot, decanted from the residue. On removing the alcohol by distillation, maltodextrin was left behind, and this was purified by further treatment with alcohol. Thus obtained, it was found to possess an optical rotatory power of $[\alpha]_{D^{33.86}} + 193.6^\circ$ and a cupric reducing power of $\kappa_{3.86} 20.7$, numbers which agree with those of a mixture of 33.9 per cent. maltose and 66.1 per cent. dextrin. Maltodextrin is absolutely unfermentable by the *Saccharomyces cerevisiae*, and cannot be separated into its constituents by further treatment with alcohol; when acted upon by a little fresh malt-extract it is *completely hydrolysed to maltose*. Here, then, is a body apparently showing by its reducing power that it contains 34 per cent. of maltose which is absolutely unfermentable by the yeast of the primary fermentation.

Hertzfeld's Maltodextrin.—Hertzfeld described in 1879¹ a body which he had found in starch conversions made with diastase at a temperature of 70° C., and to which he gave the name "maltodextrin." He regarded it as a body intermediate between achroodextrin and maltose, and assigned to it the following composition $\left\{ \begin{smallmatrix} 2\text{C}_6\text{H}_{10}\text{O}_5 \\ \text{C}_6\text{H}_{12}\text{O}_6 \end{smallmatrix} \right.$, that is, two molecules of dextrin and one molecule of glucose. He found its optical rotatory power to be $[\alpha]_j + 171.6^\circ$ and its cupric reducing power κ 23.5; but as he was under the impression that one part of glucose gave on reduction 2.362 parts of copper oxide instead of 2.205, a correction has to be applied to his κ , after which it becomes 26.7.

Since this would point to a composition of 43.70 per cent. maltose and 49.12 dextrin, the optical rotating power ought to have been $[\alpha]_j + 187.1^\circ$, but this does not conform to Brown and Morris's law of definite relation, they attribute the difference to impurities introduced into the solution by the large quantity of malt-extract which Hertzfeld used in the experiment; at the same time, they express their conviction that Hertzfeld had obtained in an impure state the body which they had similarly named maltodextrin.

Hertzfeld states that his maltodextrin was completely fermentable by *Saccharomyces cerevisiae*, but this, it was considered, might be owing to his having made use of a yeast contaminated with other forms of yeast and bacteria.

Unfermentability of Maltodextrin and the Dextrins by *Saccharomyces cerevisiae*.—The following is an example of a numerous series of experiments which were made by Brown and Morris to show that neither maltodextrin nor the dextrins could be fermented by *Saccharomyces cerevisiae*²:

The products of a starch conversion which has been separated by alcohol, and which had an optical rotatory power of $[\alpha]_{j3.86} + 191.2^\circ$, were dissolved in water; the solution had a specific gravity of 1099.5. A little yeast was then added to this solution, which contained maltose, maltodextrin, and several of the achroodextrins, and the whole left to ferment at 30° C. (86° F.). Brisk fermentation ensued, and this came to an end in three days. The solution had now an optical rotatory power of $[\alpha]_{j3.86} + 194.2^\circ$, and a cupric reducing power of $\kappa_{3.86}$ 17.7, corresponding to 29 per cent. maltose and 69.7 per cent. dextrin, and 1.3 per cent. inactive matter. When degraded with malt-extract at 60° C. (140° F.), 100 parts of the dextrin yielded 66.8 parts of maltose. The solution was now evaporated to a syrup, and sufficient water added to make a solution having a specific gravity of 1093. It then contained per 100 c.c. 6.986 grammes of maltose, 16.792 grammes dextrin, and 0.315 gramme of inactive matter. A little yeast was added, and the whole kept at a temperature of 30° C. for seven days. No trace of fermentation was to be observed,

¹ Inaugural Dissertation, Halle.

² As pure yeasts, under the Hansenian acceptance of the term, were then unknown, the *Saccharomyces cerevisiae* here mentioned is the ordinary yeast of the primary fermentation. It is stated however by Moritz and Morris ("Science of Brewing," p. 121, footnote) that on a repetition of the experiments with pure cultures, precisely the same results were obtained.

and the yeast-cells were found on examination to be shrivelled up and, to all appearance, dead. At the end of this time a slow fermentation commenced, and, coincident with this, a few cells of *Saccharomyces ellipsoideus* and *Saccharomyces Pastorianus* were detected. These latter increased in quantity, and in proportion as this increase took place, so much livelier became the fermentation. The solution was still in a state of fermentation at the end of forty days, when the experiment was discontinued. An estimation of the amount of maltose fermented away was then made (1) by observing the decrease in the specific gravity of the solution after the alcohol had been driven off by evaporation; (2) by estimating the amount of alcohol which had been formed, correction being made for the non-volatile products of fermentation in both cases.

(1) Gave a disappearance of 14.398 grammes maltose per 100 c.c.

(2) Gave a disappearance of 14.410 grammes maltose per 100 c.c.

Since the original solution contained only 6.986 grammes of maltose, the remaining 7.494 grammes must have been derived from the dextrin; for, on subsequent degradation with a little fresh malt-extract, it now yielded only 25.7 parts of maltose per 100 parts of dextrin against the 66.8 parts which it had yielded prior to fermentation.

Brown and Morris's Hypothesis of the Breaking Down of the Starch Molecule by Diastase.—The discovery of maltodextrin led Brown and Morris to a further extension of the previous views of Brown and Heron on the constitution of the starch molecule. They now regarded the starch molecule as consisting of not less than five $(C_{12}H_{20}O_{10})_3$ groups, and considered that when it was acted on by malt-extract, one of the $(C_{12}H_{20}O_{10})_3$ groups was partially hydrated and detached in the form of maltodextrin, the remaining four groups, $4(C_{12}H_{20}O_{10})_3$ forming a dextrin residue. One of the four $(C_{12}H_{20}O_{15})_3$ groups of this residue was similarly partially hydrated and detached, and the remaining three $(C_{12}H_{20}O_{10})_3$ constituted a dextrin residue of less complexity than the preceding one. This action proceeded in a similar manner until the last $(C_{12}H_{20}O_{10})_3$ group was reached, and this formed the stable dextrin of the No. 8 equation (page 153). Maltodextrin, when treated with malt-extract which had not been heated beyond $65^{\circ} C.$ ($149^{\circ} F.$), was found to be rapidly and completely hydrolysed to maltose. It was, however, possible that the hydrolysis might have taken place in two stages, but so far no indication of this had been observed.

Molecular Weight of Starch.—In a paper read before the Chemical Society in 1889,¹ Brown and Morris describe a number of experiments performed by Raoult's cryoscopic method, in which an attempt was made to ascertain the molecular weight of starch. Ordinary starch, owing to the viscid nature of its solution, was entirely unfitted for an experiment of this nature; but by operating on its near relative, soluble starch, strong indications were obtained that the molecular weight of this body was from 20,000

¹ *Journ. Chem. Soc., Trans.*, 1889, **55**, 449 and 462.

to 30,000. Not satisfied with this, an endeavour was made to ascertain the molecular weight in another way. Since starch, when hydrolysed, is invariably found to contain a fifth of its weight of a dextrin which is only further hydrolysed with extreme difficulty, it is obvious that if the molecular weight of this dextrin could be established with certainty, then that of starch would be also definitely fixed, since it must necessarily be five times as great as the molecular weight of this dextrin. Experiments made with a number of preparations of the dextrin pointed strongly to its molecular weight being 6480, corresponding to a formula of $(C_{12}H_{20}O_{10})_{20}$. The formula for soluble starch would be consequently five times as much as this, or $5(C_{12}H_{20}O_{10})_{20}$, and its molecular weight 32,400.

All the Dextrins have the same Molecular Weight.—Raoult's method was now tried upon a number of different dextrins to ascertain if they all possessed the same or different molecular weights. The following is an example of one of these experiments:

A dextrin was prepared which had an optical rotatory power of $[\alpha]_{D^{86}} + 210.7^\circ$ and a reducing power of $\kappa_{3.86} 1.4$, figures closely corresponding to those of a mixture of 2.3 per cent. maltose and 97.7 per cent. dextrin. One hundred parts of this dextrin, when degraded with fresh malt-extract, yielded 71.5 parts of maltose; consequently its mean position was between Nos. 3 and 4 of the series on p. 153. If it were a polymer, or a mixture of polymers of the lowest dextrins, its molecular weight ought to be 22,680, that is, $6480 \times 3\frac{1}{2}$. The molecular weight as deduced by Raoult's method pointed strongly to the number 6480. A number of similar experiments made with dextrins in various positions in the series shown in the table on p. 153 gave similar results; hence it was concluded that the view previously held, that the dextrins were polymeric bodies, would have to be abandoned in the face of these fresh facts. Since it was now shown that they all possessed the same molecular weight,¹ it became necessary to frame a new hypothesis in accordance with these newly-discovered facts.

Brown and Morris's Second Hypothesis of the Breaking Down of the Starch Molecule by Diastase.—This hypothesis was enunciated in a paper read before the Laboratory Club in 1890.² The authors now regarded the starch molecule as consisting of four complex dextrin groups, arranged round a fifth similar group. This last, on the breaking down of the molecule, becomes the stable dextrin of the No. 8 equation.

The first act of hydrolysis by diastase is to break up this complex molecule and liberate all the five dextrin groups, thus:

¹ O'Sullivan (*Journal of the Chemical Society*, 1879, **35**, 783) stated his belief that the first products of the breaking up of the starch molecule were a series of isomeric dextrins.

² *Trans. Lab. Club* **3**, 83.

One of five groups is, from some unknown cause, only further hydrolysed by diastase with extreme difficulty, and this forms the stable dextrin of the No. 8 equation (p. 153). The remaining four dextrin groups yield much more readily to the influence of the diastase, and are immediately broken down into a complicated series of amyloïns¹ or maltodextrins of varying composition. The extreme stages and an intermediate one are represented in the following equations :

The authors then state, "As the hydrolysis proceeds, the complex amyloïn groups break up into smaller molecular aggregations, which, however, retain all the characteristics of the amyloïns, and this goes on until the maltose stage is reached."

They adduce as examples of amyloïns which have been isolated and examined in a pure state, and the molecular weights of which have been determined, maltodextrin $\left\{ \begin{array}{l} (C_{12}H_{22}O_{11}) \\ (C_{12}H_{20}O_{10})_2 \end{array} \right\}$ (p. 158) and amyloextrin $\left\{ \begin{array}{l} C_{12}H_{22}O_{11} \\ (C_{12}H_{20}O_{10})_6 \end{array} \right\}$ (p. 83).

Hence, according to the hypothesis, there are present in all starch conversions, when carried out under the ordinary conditions of mashing employed in the brewery, free maltose, free dextrin, which is not further hydrolysed, together with a certain proportion of amyloïns of varying types.

It is assumed that the free maltose is entirely fermented away during the primary fermentation, and that the amyloïns are gradually broken down and slowly fermented away during the secondary fermentation which takes place after the beer has been stored in casks; the free dextrin persists to the last and is little, if at all, affected even on prolonged storage.

Isomaltose.—In 1891, C. J. Lintner announced the discovery amongst the products of starch hydrolysis of an entirely new sugar. Scheibler and Mittelmeier² had described, in 1889, an osazone which was obtained from a substance found in the unfermentable residue of commercial glucose, and which from its composition, was presumably the osazone of a sugar of the formula $C_{12}H_{22}O_{11}$. Shortly afterwards Emil Fischer³ obtained a substance by dissolving 100 grammes of pure glucose in 400 c.c. of hydrochloric acid of a specific gravity of 1.19, and allowing the mixture to stand at a temperature of from 16° to 20° C. (61° to 70° F.) for fifteen hours. The osazone obtained from this substance

¹ The name "amyloïn" was suggested for these bodies by Professor Armstrong. It had been proposed to terminate the name of all sugars belonging to the $C_{12}H_{22}O_{11}$ group with the syllable *on*; thus maltose would be malton; and as the dextrins already possess the termination *in*, a word ending in *oïn* would signify a compound of amyloïn with amylin, which latter name has been proposed for dextrin.

² *Ber. d. Deutsch. chem. Gesell.*, 1890, 23, 3060.

l.c., 23, 3687.

melted at 150° to 153° C., and as it resembled in several other respects maltosazone, it was named "isomaltosazone." In 1891 Scheibler and Mittelmeier¹ obtained, by fermenting a 10 per cent. solution of commercial glucose, filtering the solution, evaporating to a syrup, and adding strong alcohol, an amorphous unfermentable substance which had been previously described by Schmitt and Cobenzl under the name of "gallisin." This body, when purified by repeated precipitation from its aqueous solution by alcohol, was colourless, amorphous, and of a very hygroscopic nature. It reduced Fehling's solution, and, when heated with phenylhydrazine acetate, yielded an osazone melting at 152° to 153° C., which was readily soluble in hot water. This osazone was, they considered, identical with the isomaltosazone previously described by Fischer. In 1891 C. J. Lintner² discovered, by the aid of the phenylhydrazine reaction, a sugar in wort and beer which yielded an osazone similar to the one just described; hence he called the sugar from which it was derived "isomaltose." In a subsequent paper published by Lintner and Düll,³ the results of a further series of investigations with reference to this sugar were described. They found that after removing the maltose from the products of a starch conversion by a short fermentation conducted at 27° C. (80° F.), they were able to resolve, by repeated precipitation with alcohol, the unfermented matters into dextrin and isomaltose, but they were unable to detect even a trace of any such substance as an intermediary maltodextrin.

Preparation of Isomaltose.—Lintner and Düll prepared isomaltose in the following manner :

Two hundred and fifty grammes of potato-starch were mixed with 500 c.c. of water containing in solution 0.5 gramme of diastase; the mixture was then added to 2 litres of water having a temperature of 75° C. (167° F.). After liquefaction of the starch had taken place, another 0.5 gramme of diastase was added, and the whole kept at a temperature of 67° to 69° C. (153° to 156° F.) for three hours. The liquid, after being boiled, gave a dark brown reaction with iodine, and had an optical rotatory power of $[\alpha]_D + 170^{\circ}$. The solution was then evaporated to a syrup on the water-bath.

I. The first separation of the starch-conversion products was effected with 80 per cent. alcohol. The syrup was first saturated with alcohol, then poured into hot alcohol, the quantities being so adjusted that to every 10 parts of dry substance there were present in solution not less than 100 c.c. of 80 per cent. alcohol. The whole was allowed to stand until it became cold.

II. The clear alcoholic solution from No. I. was then decanted from the precipitated syrup which had formed, the alcohol removed by distillation, the residue made up to a 20 per cent. solution with water, and set to ferment by the addition of 2 grammes of pressed yeast for every 100 c.c. of solution. After fermenting for twenty hours all traces of maltose and glucose had

¹ *l.c.*, 24, 301.

² *Zeit. ges. Brau.*, 1891, p. 284.

³ *Zeit. angew. Chem.*, 1892, p. 268.

disappeared. The solution was then boiled, filtered, treated with animal charcoal to remove colour, once more filtered, and evaporated to a syrup.

III. This syrup was again treated with 85 per cent. alcohol in the same way as No. I., the quantities being so adjusted that for every 5 grammes of dry substance at least 100 c.c. of 85 per cent. alcohol were present.

IV. The clear alcoholic solution from No. III., when cold, was decanted from the residue, evaporated to a syrup, and treated in the same way as No. I., using this time 90 per cent. alcohol, the quantities being so arranged that for every 5, or better 3 grammes of dry substance, there were not less than 100 c.c. of 90 per cent. alcohol present. The clear liquid from this, after being decanted from the precipitated syrup, was a solution of isomaltose sufficiently pure for most purposes.

Its weight amounted to 20 per cent. of that of the original starch. The contaminating impurities, which consisted of mineral matters (ash) and acids generated during the fermentation, were only removed with great difficulty. They could only be removed by frequent solution in water and re-precipitation by alcohol.

Characters of Isomaltose.—As thus prepared, isomaltose is described as a body possessing an intensely sweet taste. Its optical rotatory power in a 10 per cent. solution is equal to $[\alpha]_D + 139^\circ$ to 140° . It has a lower reducing action on Fehling's solution; 83 per cent. that of maltose, consequently its reducing power is R. 83 or κ 50.63. Lintner did not succeed in obtaining it in a crystalline condition, but he found that when left for a considerable time under absolute alcohol, it assumed a solid form and could be rubbed to powder. It is exceedingly difficult to free isomaltose from the last traces of alcohol, since before these are completely driven off by heat the isomaltose itself begins to decompose. The weight of dry isomaltose in solution can be determined directly by placing 0.5 to 1.0 gramme of the solution in a weighing-tube with ground stopper, and keeping the whole at a temperature of 100° C. (212° F.) for some time, weighings being made at intervals of an hour until the weight remains constant. The residue assumes a yellow colour, but the decomposition is not sufficiently advanced to affect the accuracy of the result. Isomaltose is of an exceedingly hygroscopic nature. It was said to be slowly fermented by yeast, and, when acted upon by diastase, to be completely converted into maltose.

The following is the view adopted by Lintner and Düll with regard to the transformation of starch under the influence of diastase, commencing with amyloextrin (soluble starch):

- I. $(C_{12}H_{20}O_{10})_{54} + 3H_2O = 3[(C_{12}H_{20}O_{10})_{17} \cdot C_{12}H_{22}O_{11}]$.
- II. $3[(C_{12}H_{20}O_{10})_{17} \cdot C_{12}H_{22}O_{11}] + 6H_2O = 9[(C_{12}H_{20}O_{10})_5 \cdot C_{12}H_{22}O_{11}]$.
- III. $9[(C_{12}H_{20}O_{10})_5 \cdot C_{12}H_{22}O_{11}] + 45H_2O = 54C_{12}H_{22}O_{11} = \text{Isomaltose.}$
- IV. $54C_{12}H_{22}O_{11} = \text{Maltose.}$

They did not consider that these stages succeeded one another in regular order, but that the action proceeded irregularly, some of the amyloextrin molecules being much more rapidly broken down than others; consequently

by the time some of these had reached the stage of isomaltose and maltose, others might only be in the earlier stages of degradation. In this way it was sought to explain the simultaneous presence of maltose and the higher dextrans in high-angled transformations.

Schiffner's Experiments on the Conversion Products of Starch by Diastase.—At the instigation of Lintner, Anton Schiffner carried out a series of experiments on this subject. These were embodied in an "Inaugural Dissertation"¹ delivered at the University of Kiel in 1892.

He first attempted to reproduce the maltodextrin of Brown and Morris (p. 158), and followed the directions given for its preparation² as far as possible; but complains that in some points these are somewhat vague. A starch transformation was conducted at a temperature of 60° to 65° C. (140° to 149° F.), using 500 grammes of potato-starch and 0.416 gramme of diastase. The action was allowed to proceed for fifty minutes, when the conversion showed an optical rotatory power of $[\alpha]_D + 180^\circ$. The solution was then boiled and filtered. The osazone obtained from the filtrate sintered at 145° C. and melted at 152° C. This, according to the author, showed that the whole of the transformation products consisted of isomaltose and dextrin, hence the subsequent fermentation for the removal of maltose might have been omitted. To be assured that any traces of maltose, the presence of which might not have been rendered evident by the osazone, were removed, 5 grammes of freshly washed pressed yeast were added to the solution and fermentation allowed to proceed for twenty-four hours at the ordinary temperature. The solution was then filtered, evaporated to a syrup, and poured while hot into so much hot absolute alcohol that the quantity of alcohol amounted to 90 per cent. of the whole mixture.

After this had been kept boiling for two days under a reflux condenser the hot alcoholic solution was decanted from the precipitated syrup, filtered, and the alcohol distilled off. Water was then added to the residue, and the whole evaporated to a syrup. This syrup contained 24 grammes of dry substance, which had an intensely sweet taste, and an optical rotatory power of $[\alpha]_D + 142^\circ$; it yielded an osazone which sintered at 142° and melted at 152° C. This product consisted, therefore, of nearly pure isomaltose. A second experiment gave similar results. No trace of a substance similar to Brown and Morris's maltodextrin was observed.

In another experiment, where 1000 grammes of potato-starch were used, a conversion was obtained which, after being boiled and filtered, had an optical rotatory power of $[\alpha]_D + 152.01^\circ$ and a reducing power of κ 43.4 (R. 71.12). It was fermented with yeast, and filtered, treated with animal charcoal, and evaporated to a syrup. This syrup was now fractionated with alcohol, much after the manner described before in Lintner's experiments, with the result that the products of the starch conversion were entirely separated into dextrin and isomaltose, no trace of any intermediary maltodextrin being observed.

¹ Published by Schmidt and Klaunig, Kiel.

² *Journ. Chem. Soc., Trans.*, 1885 47, 562.

The experiment was so arranged that nothing except the first fraction, which consisted principally of albuminous and mineral matters, was lost.

Another experiment was made with 1000 grammes of potato-starch, in which Hertzfeld's directions for obtaining *his* maltodextrin were exactly observed. The whole of this was fractionated with alcohol, and was found to be separable into dextrin and isomaltose, no trace of maltodextrin being observed. Schifferer considers that Brown and Morris's maltodextrin was a mixture of about 67 per cent. dextrin and 33 per cent. isomaltose, and Hertzfeld's a mixture of about 74 per cent. isomaltose and 26 per cent. dextrin.

Compound Nature of Diastase.—Cuisinier held the view that malt diastase consisted of two enzymes, which he termed respectively "maltase" and "dextrinase." He considered that the latter alone was able to attack starch, which it first liquefied and then converted into dextrin. The maltase then acted on the dextrin and transformed it into maltose. Weijsmann¹ further elaborated this theory of two enzymes, but took a somewhat different view of their respective actions. In his opinion, the maltase yields maltose, and a high erythro-dextrin, which he terms "erythrogranulose"; whilst the dextrinase produces maltodextrin, but no maltose. As the action proceeds, the maltase attacks the maltodextrin and transforms it into maltose, the dextrinase meanwhile converting the erythro-granulose into achroodextrin, according to the following scheme :

This action was demonstrated by a number of very ingenious experiments. Beyerinck² holds a somewhat similar view: according to this, diastase consists of maltase and granulase; the former is held to convert a portion of the starch first into erythro-dextrin, then into maltose, the granulase simultaneously converting another portion into maltodextrin, and afterwards into maltose.

Scheibler and Mittelmeier on the Dextrins.—Since the time that the properties of the gummy substance found in all starch transformations was first critically examined by Biot and Persoz in 1833, who, finding that it rotated the polarised ray powerfully to the right, named it "dextrin," this body, or rather mixture of bodies, has formed the theme of numerous investigations and of much discussion. Even at the present time it cannot be said that any absolutely definite conclusion has been arrived at as to the real character of these bodies. The question as to whether they possess reducing powers or not has long been a moot point. Brown and Morris believed they had obtained a non-reducing dextrin by the action of alkaline mercuric

Zeit. f. das ges. Brau., 1890, p. 186.

² *Centralblatt f. Bakt. u. Parasitenkunde*, 1895, p. 221.

cyanide on a crude dextrin. This body was, however, in all probability a carboxylic acid (see Scheibler and Mittelmeier, p. 78; Brown and Millar, p. 177). It has always been held by the Continental chemists that there were several dextrans, all of which possess a greater or less reducing power, and, according to the light of recent research, this seems to be the more correct view.

In 1890 Scheibler and Mittelmeier¹ pointed out the close analogy between the behaviour of the dextrans and that of the sugars which contain the CHO group. For this demonstration they employed commercial dextrin purified by treatment with alcohol. The purified preparation was coloured yellow or brown by heating with a caustic alkali, and it also reduced Fehling's solution, both of which actions are characteristic of the sugars which contain the CHO group. They also succeeded in obtaining the phenylhydrazones and phenylosazones of the dextrans, both of which were soluble in cold water. Since the osazones of the monosaccharides are insoluble in water, either cold or hot, and those of the disaccharides are soluble in hot water, it was naturally to be expected that the osazones of the more complex aldoses would be soluble in cold water. By acting on a dextrin with nascent hydrogen, they were able to obtain a body having the characteristics of an alcohol. By the action of bromine on the dextrans bodies were formed which were acid to litmus-paper, and which decomposed calcium carbonate with liberation of carbon dioxide; evidently the CHO groups of these bodies had been converted into the COOH group; that is to say, the dextrans had been changed into their corresponding carboxylic acids. These acids could be hydrolysed by diastase or by an acid, just as the corresponding maltobionic acid could. They considered these bodies were the same as those which Bondonneau produced by means of cupric chloride and caustic soda, and which Wiley obtained when he employed alkaline mercuric cyanide, and likewise by Brown and Morris when they used this latter reagent for removing what they believed to be the last traces of a reducing sugar from dextrin. In all these cases the assumption that the dextrin remained unattacked was erroneous; it suffered the alteration alluded to above, and, as a consequence, lost its property of reducing Fehling's solution. From these facts they concluded that the non-reducing dextrin of Brown and Morris has no existence. The only objection which can be urged against these experiments of Scheibler and Mittelmeier is that the dextrans operated upon were not obtained from starch by the action of diastase, but Brown and Morris have also adduced an amylo-dextrin, produced by the action of acid, in support of the amyloin theory.

Lintner and Düll on the Dextrans.—These investigators, after a long and searching examination into the products of starch transformation by diastase,² in which the various bodies were separated by long and tedious fractionation with alcohol of different strengths, and in which the properties of the isolated bodies were controlled by their rotatory and reducing powers were able to isolate the following substances:

¹ *Ber. d. Deutsch. chem. Ges.*, 1890, **23**, 3060.

² *Ibid.* 1893, **26**, 2533.

Amylodextrin.—This is obtained, on precipitating its aqueous solution with alcohol, as a loose white powder, slightly soluble in cold water, readily soluble in hot, and separates from a 20 to 30 per cent. solution in sphæro-crystals. It is insoluble in 40 per cent. alcohol, has a rotatory power of $[\alpha]_D + 196^\circ$, and does not reduce Fehling's solution. It gives a deep blue colour with iodine, and is the principal constituent of soluble starch. They regard its composition as $(C_{12}H_{20}O_{10})_{54}$.

Erythrodextrin.—This body separates from hot solutions of dilute alcohol in sphæro-crystals, is readily soluble in water, very slightly so in 50 per cent. alcohol. Its rotatory power is $[\alpha]_D 196^\circ$, its reducing power is R 1—i.e., 1 per cent. that of maltose, and it gives a reddish-brown colour with iodine. Its molecular weight was found to be 5786. The authors consider that its composition is $(C_{12}H_{20}O_{10})_{17} \cdot C_{12}H_{22}O_{11}$, and that it arises from the hydrolysis of three molecules of amylodextrin, thus :

Achroodextrin I.—This dextrin forms deliquescent sphæro-crystals, is very soluble in water, hardly soluble in 70 per cent. alcohol, and gives no coloration with iodine. It has a rotatory power of $[\alpha]_D + 192^\circ$, and a reducing power of R 10—i.e., 10 per cent. that of maltose. Its molecular weight was found to be 1963. They regard its composition as $(C_{12}H_{20}O_{10})_5 \cdot C_{12}H_{22}O_{11}$, and consider that it arises from the hydrolysis of three molecules of erythrodextrin, thus :

Achroodextrin II. was first found in the transformation products of starch hydrolysed with oxalic acid, and afterwards, though only in small quantities, in those obtained by the action of diastase. It has a rotatory power of $[\alpha]_D + 180^\circ$, and a reducing power equal to 24 per cent. that of maltose (R 24). Its molecular weight is given as 979.

Ost on the Dextrins.—This observer¹ isolated from those portions of a starch transformation soluble in 80 per cent. alcohol, from which the maltose had been removed by long-continued fractionation with 95 per cent., 90 per cent., and finally 85 per cent. alcohol, a dextrin having a rotatory power of about $[\alpha]_D 180^\circ$, together with maltose, and a considerable amount of other products which were evidently mixtures. He also found that the osazone test afforded a safe criterion of the presence of maltose in mixtures of this nature when present in quantities above 5 per cent. The above dextrin, when precipitated by alcohol and dried, formed a snow-white powder, not in the slightest degree deliquescent. It had a rotatory power of $[\alpha]_D + 180-183^\circ$, and a reducing power equal to 28-33 that of maltose (R 28 to 33). On analysis it yielded figures closely agreeing with the formula $C_{36}H_{62}O_{31}$. Ost believed that it was a definite compound, that it was essentially the same body as Lintner and Düll's achroodextrin II. $[\alpha]_D + 180^\circ$ (R = 24), and that it was contained in the maltodextrin of

¹ *Chem. Zeitung*, 1895, p. 1501.

Brown and Morris $[\alpha]_{D_{3.86}} 171^\circ$ ($R = 34$). Ost considers that in the preparation of amorphous bodies like the dextrins absolute purity cannot be expected, as it is extremely probable that they may undergo some little change during the frequent evaporations and treatment with alcohol to which they are subjected in the course of isolation.

Investigations of Ling and Baker on Isomaltose.—Much doubt has been thrown on the existence of isomaltose by several observers. The first to bring this subject under notice were Ling and Baker, in a paper read before the Chemical Society.¹ Working with diastase prepared from air, dried malt, and following Lintner and Düll's directions, they obtained a substance having an optical rotatory power of $[\alpha]_{D_{3.86}} + 144.5^\circ$ and a reducing power of $R_{3.86} 79.64$. Its osazone, after being recrystallised from water, had a melting-point between 160° and 170° C. The substance itself, after being kept in a desiccator for some weeks, became brittle and could be crushed to powder; it was very hygroscopic. Viewed under the microscope, it was seen to contain crystals resembling those of maltose. It had a sweet, but by no means an intensely sweet taste. Both from the results of its ultimate analysis, and of the determination of its molecular weight by Raoult's method, figures indicative of a disaccharide were obtained. When submitted to the action of diastase, the optical rotatory power was reduced to $[\alpha]_{D_{3.86}} + 131.2^\circ$ and the reducing power increased to $R_{3.86} = 94.5^\circ$.² As had been previously observed by Hiepe, they found that these changes were not accompanied by an alteration in the specific gravity of the solution. Mixed with ordinary brewer's yeast and kept at a temperature of 68° to 86° F., the substance fermented, though not vigorously. They came to the conclusion that the body which had been termed "isomaltose" by Lintner was a mixture of maltose and of a simple dextrin having the formula $C_{12}H_{20}O_{10} + H_2O$, a composition which would account for the absence of a rise in the specific gravity of its solution under hydrolysis. It should be observed that the substance prepared by Ling and Baker yielded an osazone of higher melting-point than the so-called isomaltosazone obtained by Lintner.

In some further experiments where diastase obtained from high-dried malt was employed, Ling and Baker succeeded in obtaining a body which yielded an osazone having a melting-point identical with that of Lintner's isomaltosazone. An ultimate analysis of this substance yielded figures closely agreeing with those required for the osazone of a trisaccharide. From this they concluded that the body which yields the osazone melting at 151° C. was a trisaccharide. They also noticed that in starch transformations conducted with diastase from high-dried malt a certain amount of glucose was produced, but never when diastase from low-dried malt had been employed.

In a second paper³ Ling and Baker stated that all the experiments they had made up to that time appeared to favour the view previously put forward,

¹ *Journ. Chem. Soc., Trans.*, 1895, 43, 702.

² This result, corrected for the error of 5 per cent. in Wein's table, is $R 98.9$.
Journ. Chem. Soc., Trans., 1895, 43, 739.

that isomaltose contained a simple dextrin, $C_{12}H_{20}O_{10} + H_2O$, which might be an intermediate product between the higher dextrans and maltose. They found that, by recrystallising the osazone derived from their preparation of isomaltose from water at $35^\circ C.$, portions crystallised out having considerably lower melting-points than those portions which afterwards formed at the ordinary temperature; and, by successive recrystallisations in this way, they were able to obtain an osazone melting at 182° to $183^\circ C.$ which closely resembled maltosazone in appearance. A fraction obtained in another experiment, which melted at $160^\circ C.$, yielded results on analysis closely agreeing with the composition of a disaccharide, consequently they regarded the fractions which melted between 150° and $160^\circ C.$ as impure maltosazone. In fact, they were able to obtain a similar osazone by heating mixtures of maltose and dextrin with phenylhydrazine acetate, but this impure osazone could be readily purified by recrystallisation from hot water. They found that when a mixture of maltose and glucose was treated with phenylhydrazine acetate, the osazones of both these sugars could be separated in a state of purity. In working with diastase from kiln-dried malt it was noticed that a small quantity of glucose was invariably formed, and this led to their trying the action of diastase obtained from this kind of malt upon maltose, when it was found that glucose was produced.¹

In transformations of starch conducted with the diastase from kiln-dried malt, they were always able to detect the presence of glucose by the formation of its osazone; but the greater part of the osazone from the products of the transformation, soluble in 90 per cent. alcohol, melted at $151^\circ C.$, and had the appearance characteristic of Lintner's isomaltosazone. They gave, however, analytical values intermediate between those required for the osazone of a disaccharide and that of a trisaccharide. From this, and from the fact that apparently no maltosazone was obtained from the product of these conversions, they were at first inclined to think that no maltose was present, but afterwards they were able to show the presence of maltose in similar products. It appeared, therefore, as if a trisaccharide was one of the products of the action of kiln-dried malt on starch; attempts, however, to isolate any other crystalline sugar than maltose entirely failed. Since this osazone of a trisaccharide was only obtained in those cases where glucose was present, they surmised that the trisaccharide might be formed by the condensation of a molecule of the simple dextrin with one of glucose under the influence of phenylhydrazine.

The "isomaltose," prepared by means of the diastase from low-dried malt and glucose, yielded a mixture of glucosazone and a soluble osazone; the latter, after recrystallisation, melted at 150° to $160^\circ C.$, and on analysis appeared to be a mixture of the osazones of a di- and a tri-saccharide, a result which might be expected, considering that maltose is a constituent of

¹ Subsequent experiments have proved that glucose is only produced when certain kilned malts are used, probably those which have been heated for some time in presence of a considerable amount of moisture on the kiln. See Ling and Davis, p. 181.

“isomaltose.” Microscopical examination of the osazone showed that it consisted almost entirely of spherical aggregates of needles, but, under a higher magnification, flat ribbon-like crystals could also be detected.

Brown and Morris on Isomaltose.—Messrs. Ling and Baker's paper was shortly followed by one from Messrs. Brown and Morris,¹ also throwing doubt on the existence of isomaltose. They first point out that this body does not conform to their “law of definite relation,” which they consider to be the most valuable criterion of the purity of starch-transformation products. Lintner's isomaltose has an optical rotatory power of $[\alpha]_D + 140^\circ$ and a reducing power of $R = 80$, equivalent to $[\alpha]_J + 156^\circ$ and $\kappa 48.8$. This optical rotatory power, according to their law, requires a reducing power of $\kappa 55.3$, and a reducing power of $\kappa 48.8$ requires an optical rotatory power of $[\alpha]_J + 163.2$. After an elaborate investigation of a starch transformation conducted on the method described by Lintner, they came to the conclusion that isomaltose was not a chemical entity, but that by fractionation with alcohol, by decomposition, and by fermentation it could be split up in such a manner as to indicate that it was a mixture of maltose and bodies of the amyloïn type. They considered that isomaltosazone was nothing but maltosazone, modified in its crystalline habit and melting-point by the presence of some impurity; and they were able to compel maltosazone to crystallise in the isomaltose form by the addition of a trace of an amorphous substance, which was produced during the treatment of those fractions of a starch conversion supposed to contain “isomaltose” with phenylhydrazine acetate, and which they regard as a product of the action of this reagent with substances of the maltodextrin or amyloïn class. On fermenting a solution of isomaltose, they found 50 to 60 per cent. of this body fermentable, and from a calculation of the optical rotatory and reducing powers of the solution before and after fermentation, they concluded that the portion fermented is simply maltose, and that the unfermented portion approaches very closely in composition that of an amyloïn containing two amylon groups and one amylin group. They considered that the only *crystallisable* body formed in starch transformations by diastase is maltose, and they pointed out that a study of the osazone alone cannot be relied on as a sufficient means for identifying a sugar.

Lintner's Rejoinder.—This author urged² that Brown and Morris set about their course of investigation in an incorrect way, and could consequently only arrive at erroneous conclusions. They evidently worked with impure materials, and could in this way readily obtain osazones resembling that of isomaltose. That they had a mixture of maltose and dextrin in their hands was shown by the isolation of the maltose which they accomplished on further fractionation, and also by the residue left behind after fermentation, which had a higher optical rotatory power and a lower reducing power than isomaltose, and was, therefore, a dextrin. He pointed out further that it was a very unscientific proceeding to endeavour

¹ *Journ. Chem. Soc., Trans.*, 1895, 43, 702.

² *Zeit. f. Brau.*, 1895, p. 233

to form isomaltose by making mixtures of maltodextrin and maltose, because naturally mixtures of such a nature would yield osazones having a lower melting-point than maltosazone. He himself had observed the same phenomena with mixtures of maltose and isomaltose, but he had never found it possible to transform isomaltosazone into the well-characterised maltosazone, nor to raise its melting-point.

Ost's Investigations on Isomaltose.—This author made an attempt¹ to prepare isomaltose according to the directions given by Lintner and Düll. The fractions obtained with 85 to 90 per cent. alcohol, and which had a rotatory power of about $[\alpha]_D^{140^\circ}$, were collected, dissolved in methylic alcohol, and separated into three or four portions. The treatment with methylic alcohol was found unsatisfactory, since fractions obtained in this way, and which had a rotatory power of $[\alpha]_D^{140^\circ}$, did not prove to be homogeneous, and could be further separated by ethylic alcohol. A successful result could be obtained by the employment of ethylic alcohol, but the operation was a most tedious one, on account of the ready solubility of maltose in dextrin syrups, and of the dextrans in maltose solutions. Owing to this, the further prosecution of the process was handed over to Herr Ulrich, who was unable to detect the presence of isomaltose amongst the starch-transformation products.

Ost then tried another method for removing the maltose. It consisted in evaporating the alcoholic extracts with sufficient water to bring the maltose into the hydrated condition, in which state it could be readily induced to crystallise; since isomaltose is said to be uncrystallisable, it was thought that this method would afford a ready means of separating the two bodies. According to a statement made by Soxhlet, crystallised maltose is but slightly soluble in methylic alcohol, but maltose and dextrin syrups are soluble in all proportions, hence, by bringing maltose into the crystalline condition, it should be possible to remove it completely in this way. After the maltose had been removed from the products of a starch transformation in this manner, there only remained small quantities of syrup, which had a rotatory power of less than $[\alpha]_D^{145^\circ}$. These were tested for isomaltose by the phenylhydrazine method. Osazones, very similar to those said by Lintner and Düll to be characteristic of isomaltose, were obtained from them; but, on further study, they proved to be maltosazone. This body was found to be of very changeable nature, it could be readily made to alter its crystalline form, melting-point, &c., by recrystallisation, by heating, or by a difference in the conditions under which it was formed. Especially interesting is the fact (first observed by Ling and Baker) that mixtures of maltose and dextrin, which latter body itself yields no crystalline osazone, when treated together yield a crystalline osazone simulating isomaltosazone.

Experiments were also made in which part of the starch-transformation products had been removed by fermentation with yeast. In those where the whole of the maltose had been removed, no osazone could be obtained; those

¹ *Chem. Zeitung*, 1895, p. 1501.

in which a portion of the maltose had been left unfermented yielded osazones similar to isomaltosazone.

Ost came to the conclusion that isomaltose has no real existence, and that the isomaltosazone of Lintner and Düll is simply modified maltosazone. He considers that the altered rotatory and reducing power, the uncrystallisability, and the browning of the original substance which ensued on heating, simply depended on impurities, and the reason why Lintner and Düll did not obtain pure maltose was to be attributed to their using methylic alcohol in the fractionation.

Jalowetz¹ also came to substantially the same conclusion as Ost on this subject.

Ost's Conclusions with Regard to the Action of Diastase upon Starch.—After an investigation into the products of starch transformation by diastase,² to which reference has been already made (p. 172), Ost concludes that starch itself has the composition $(C_{12}H_{20}O_{10})_n$, the n indicating a large but as yet unknown number; that the first products of the action which possess a reducing power, though their rotatory power is either unaffected or affected in a degree so small as to be unrecognisable, contain chemically combined water, and that dextrins of the composition $(C_{12}H_{20}O_{10})_n$ have no existence. He considers that the non-reducing dextrin of Brown and Morris is non-existent, the substance obtained by treatment with alkaline mercuric cyanide consisting, as had been previously shown by Scheibler and Mittelmeier, of the carboxylic acids of the dextrins. He points out that O'Sullivan was never able to obtain a dextrin entirely free from reducing power, that the law of definite proportion between the rotatory and reducing powers enunciated by the English observers is not always fulfilled even in their own experiments, and that it is certainly not in the achroodextrin II. which he himself had prepared. The method of determining the reducing power employed by the English chemists is, Ost says, a fallacious one, and so is the method of estimating the quantities of the analysed material from the specific gravity of the solution instead of by actually determining the weight of the matter in solution. Though this may suffice for approximate determination it is insufficient for the deduction of important laws. The theory of Brown and Heron arose at a time (1879) when the reducing power of the dextrins was solely attributed to the maltose with which they were contaminated. Ost considers that, after maltodextrin and amylo-dextrin had been recognised by Brown and Morris as individual substances with independent reducing powers, the old hypothesis, instead of being altered to the amyloïn theory, should have been entirely discarded, since it is neither in harmony with the real facts nor is it without internal contradictions. He was unable to decide whether the different erythro- and achroo-dextrins of Lintner and Düll are individual substances, but he considered they were obtained in as pure a condition as our present means would allow. Ost believed the molecular weight determinations, especially those of the higher dextrins, were of a very doubtful

¹ *Chem. Zeitung*, 1895, p. 2003.

² *Ibid.* p. 1501.

value, an opinion also shared by Kuster. He was disposed to regard the erythrodextrins not as individual substances, but as mixtures of starch and achroodextrin, in accordance with the view which had been put forward by Musculus and Meyer, who found that achroodextrin, to which a half per cent. of soluble starch had been added, gave a red colour with iodine. Should this prove to be the case, then all the dextrins would be achroodextrins.

Mittelmeier's Views on Starch Degradation.¹—According to this observer, the first action of diastase on the starch molecule is to split it up into two amylo-dextrins, which exhibit considerable differences in their further behaviour with diastase, one of them being much more resistant to its action than the other. By the time taken for the first to reach the stage in which it gives a coloured red with iodine, the second has passed through all the intermediary phases of starch degradation down to maltose. In this way an explanation is readily afforded of the simultaneous presence of the highest dextrins and maltose in the same starch conversion. That the two amylo-dextrins differ chemically is shown by the fact that the dextrins which they respectively yield differ. Mittelmeier thinks it probable that there is a greater number of chemically different dextrins than has hitherto been assumed.

The erythrodextrin which forms the more quickly he terms the primary; it is distinguished from the secondary by its comparative insolubility in cold water, and by being precipitated from its hot solution on cooling as a pulverulent precipitate, which increases on standing. It is insoluble in cold water, but soluble in water having a temperature of 60° to 65° C. Both the primary and secondary erythrodextrins reduce Fehling's solution and form compounds with phenylhydrazine, thus showing their aldehydic nature. Their optical rotatory power is about $[\alpha]_D + 170^\circ$. Under the influence of diastase, a primary achroodextrin arises from the primary erythrodextrin, which reduces Fehling's solution still more powerfully than the dextrin from which it is derived. It is soluble in water, but can be precipitated from its aqueous solution by the addition of alcohol, when it assumes the form of star-shaped crystalline aggregates. On further hydrolysis it yields maltose. A secondary archroodextrin is also derived from the secondary erythrodextrin. This is characterised by its different behaviour with diastase, and also by the fact that its hydrolytic products yield jelly-like osazones.

Metamaltose.—The osazone of this body was found by Mittelmeier¹ amongst the jelly-like substances which are invariably formed when the products of a starch transformation are treated with phenylhydrazine acetate (see last paragraph). The secondary achroo- and erythro-dextrins when treated with malt-extract yield an abundance of this substance, whilst the primary ones under similar treatment do not. These jelly-like osazones are only purified with the greatest difficulty, and on analysis gave figures corresponding with those of the osazone of a disaccharide. The osazone when heated began

¹ *Zeit. ang. Chem.*, 1895, p. 552.

to sinter at 120° C., melted at 145° to 148° , and commenced to decompose at 160° to 165° C. It evidently differed considerably from the osazones hitherto obtained from starch-transformation products, and as the body which yields it is produced in considerable quantity by diastatic action, and cannot be found in beer, Mittelmeier thinks that it probably plays an important part in fermentation.

Prior's Achroodextrin No. III.—This body was isolated in 1896¹ from the products of a starch transformation which gave the reaction for erythro-dextrin, and from which the more easily fermentable bodies had been removed by fractionation with alcohol. It had an optical rotatory power of $[\alpha]_D + 172.1^{\circ}$ and a reducing power of R 42.5.² It was considered that this substance probably had the composition $(C_{12}H_{20}O_{10})_2 + H_2O$. It was fermentable with difficulty, and then only partially by the Froberg or Saatze yeast, the fermentation being carried to a somewhat higher degree by the former. Mixed with 20 per cent. of maltose, it yielded an osazone which exactly resembled the so-called isomaltosazone.

Further Investigations of Ling and Baker.—In a third paper read before the Chemical Society,³ these chemists gave the results of an examination in great detail of the products of the limited action of diastase on potato-starch paste at 70° C. They separated from these products maltose and the following maltodextrins, the formulæ of all of which were established by elementary analysis and by determinations of the molecular weight by the cryoscopic method.

Maltodextrin- α , $C_{36}H_{62}O_{31}$, probably identical with the maltodextrin of Brown and Morris (see p. 158). The optical rotatory and cupric reducing powers of this compound, observed by Ling and Baker, were $[\alpha]_D + 180^{\circ}$; R 32.81.

Maltodextrin- β , $C_{24}H_{42}O_{21}$, probably identical with Prior's "achroodextrin III," and having the constants $[\alpha]_D + 171.6^{\circ}$; R 43.

A substance, $C_{12}H_{22}O_{11}$, isomeric with maltose, and obtained from the unfermentable residue of the particular fraction described as "isomaltose" by Lintner. It had the constant $[\alpha]_D + 156$; R 62.5, and the authors suggested that it might consist of a simple "dextrin" (maltodextrin), $C_{12}H_{20}O_{10} + H_2O$, the existence of which their work had previously foreshadowed. Inasmuch as the authors' preparation gave a small amount of crystalline osazone, it may have contained a little maltose. (Compare, however, Syniewski, p. 178.)

Researches of Brown and Millar.—These chemists published a series of papers in 1899.⁴ They showed that when the maltodextrin isolated by Brown and Morris (see p. 158) is purified, it has the constants $[\alpha]_D 181-183^{\circ}$; and R 42-43. When carefully oxidised with mercuric oxide and barium hydroxide until its cupric reducing power disappears, the greater part of the product is obtained as the barium salt of a definite and complex carboxylic

¹ *Bayer, Brauer.*, 1896, p. 157.

³ *Journ. Chem. Soc., Trans.*, 1897, 71, 509.

² *Ibid.* 1896, p. 385.

⁴ *Ibid.* 1899, 75, 286-337.

acid which is provisionally called "maltodextrinic acid A." This has been isolated. Its properties correspond to $[\alpha]_D^{19.2-3}$ and R 0, and its calcium salt contains 2.4 per cent. of calcium. The acid is hydrolysed both by diastase and by dilute acids. Under the action of the diastase, it yields 40 per cent. of maltose and 60 per cent. of another carboxylic acid of less complexity; this is maltodextrinic acid B, the calcium salt of which contains 3.8 per cent. of calcium. When hydrolysed by dilute oxalic acid maltodextrinic acid A yields 85.8 per cent. of *d*-glucose and another still simpler acid which is shown to be a C_5 -derivative. Maltodextrinic acid B when hydrolysed by oxalic acid also yields the same C_5 -acid and 67.7 per cent. of glucose.

The final C_5 -acid corresponds in composition to $C_5H_{10}O_6$ and appears to be a normal carboxylic acid, derived from a pentose. This acid can also be prepared by the oxidation of maltose with mercuric oxide. It has many properties in common with those of xylonic acid. Taking the constitutional formula for maltose as :

maltodextrin may be regarded as made up of three such molecules with the elimination of $2H_2O$. Written in an abbreviated form, the formulæ of maltodextrin, and of maltodextrinic acid A, may be expressed thus :

The sign $\diagup \diagdown$ denotes the open carbonyl.

The hydrolysis of maltodextrinic acid A in two successive steps by diastase and acids respectively may be represented in the following manner :

(1) Diastase hydrolysis

(2) Acid hydrolysis

According to Brown and Morris, when starch is transformed by an active diastase, such as that derived from an air-dried malt at temperatures below 60°C ., the reaction proceeds with great rapidity until a resting-stage is reached corresponding to $[\alpha]_D^{10.5}$ R 80 for the mixed products of change.

Brown and Millar point out that at this stage these products consist of maltose and a dextrin, which latter, comparatively speaking, is very resistant to the further action of the hydrolytic agent. They obtained this dextrin with optical and reducing properties corresponding to $[\alpha]_D^{20}$ 195–195.7° and R 5.7–5.9. The reducing power is a property inherent to the dextrin and is not due to admixture with a second reducing substance. This is shown by oxidising the dextrin with mercuric oxide and baryta, a definite dextrinic acid being produced, which is a complex carboxylic acid with distinct though feeble acid properties. It forms a calcium salt containing 0.31 per cent. of calcium, and under the influence of acid hydrolysis yields *d*-glucose and a residual C_5 -acid, identical with that obtained by the complete acid hydrolysis of the two maltodextrinic acids already described.

Dextrinic acid and its salts and also the dextrin from which it is derived are but very slowly hydrolysed by diastase, and yield after many hours' treatment at 50–60° C. maltose and glucose in about equal proportions. It is stated that the available evidence all points to the conclusion that the stable dextrin and the derived dextrinic acid are built up of C_6 -, and not, as in the case of maltodextrin, of C_{12} -groups.¹ The dextrin molecule may be empirically regarded as made up of $39C_6H_{10}O_5$ groups in combination with a terminal $C_6H_{12}O_6$ group; or, in other words, as a condensation of forty glucose molecules with the elimination of $39H_2O$. Brown and Millar, therefore, ascribed the following constitutional formulæ to the dextrin and acid respectively:

Non-reducing Dextrins.—It should here be mentioned that Pottevin² concludes from his experiments that the maltodextrins are mixtures of maltose and a non-reducing dextrin. He finds when maltose is mixed with certain dextrins it cannot be fermented. Against this view it may be pointed out that the maltodextrins do not give crystallisable osazones unless mixed with sugar. J. Moreau³ obtained non-reducing dextrins by fractionally precipitating the crude substances with baryta solution and alcohol. In this case there is the possibility that oxidation to the maltodextrinic acid may occur.

Syniewski's Investigations.—V. Syniewski in two most important papers⁴ points out that starch can be hydrolysed in two ways, either so as to yield substances which do *not* reduce alkaline copper solutions (carbinol hydrolysis), or those which do reduce alkaline copper solutions (carbonyl hydrolysis). His experiments show that the simplest product of carbinol hydrolysis of starch is a substance which he terms "amylogen." It has the

¹ Ling has shown, however (see p. 182), that all the products of the action of diastase on starch, with the exception of maltose, yield a certain proportion of glucose when isolated and acted upon with a further quantity of diastase.

² *Annales de l'Institut. Pasteur*, 1899, **13**, 665, 728, and 796.

³ *Annales de la Soc. Roy. des Sciences Méd. et Nat. de Bruxelles*, 1904, **64**.

⁴ *Liebig's Annalen*, 1900, **309**, 282; 1902, **324**, 212.

molecular formula, $C_{54}H_{96}O_{48}$, and is obtained by the action of sodium peroxide on potato-starch. He finds that by heating starch paste in an autoclave at 140° C., carbinol hydrolysis occurs and proceeds as far as amyloextrin, $C_{216}H_{372}O_{186}$ (see below). The products of hydrolysis of starch by malt-extract always possess a certain amount of reducing power; that is to say, it is not possible to restrict the action of malt-extract entirely to carbinol hydrolysis.¹

Action of Heated Malt-Extract on Starch.—A solution of starch (amyloextrin), prepared by heating potato-starch paste in an autoclave at 140° C., in which the carbon in the dissolved substance contained in a definite volume had been estimated and calculated to amylogen, was treated (at ordinary temperature?) with malt-extract previously heated at 78° C. for 18 minutes. At the end of 216 hours the solution gave no coloration with iodine and contained exclusively a single product identical with the maltodextrin of Brown and Morris, the achroodextrin II of Lintner and Düll, and the maltodextrin- α of Ling and Baker. After 2832 hours hydrolysis had proceeded to a stage at which the apparent maltose, calculated on the original amylogen contents of the solution, was 50.12 per cent. When malt-extract, heated to 77° C., was used as converting agent, the maltodextrin stage was reached in six hours.

Syniewski denotes this maltodextrin *final dextrin II* ("*Grenz dextrin II*"). He states that it is an amorphous substance, having a sweetish taste, somewhat readily soluble in boiling 80 per cent. alcohol, but sparingly so in 90 per cent. alcohol; that it has a specific rotatory power of $[\alpha]_D + 179.36^{\circ}$ at 20° C., and a cupric reducing power of R 30. Elementary analyses and determinations of the molecular weight by the cryoscopic method, established the formula $C_{36}H_{62}O_{31}$ (compare Ling and Baker (p. 175)). Syniewski does not consider the observation of Brown and Millar² that this compound has the constants $[\alpha]_D + 181-183^{\circ}$; R 42-43 $^{\circ}$ proved.³ Final dextrin II can be hydrolysed by treatment with either unheated or heated malt-extract, the difference between the action of the two being that the latter is slower. When hydrolysis is continued until the reducing power of the dissolved solids is R 60, the product consists mainly of a compound $C_{24}H_{42}O_{31}$, which Syniewski describes as γ -maltodextrin, and which in the pure state has the constants $[\alpha]_D + 172.17^{\circ}$, R 42.7. It is an amorphous substance having a pleasant and sweet taste, readily soluble in 80 per cent. alcohol, sparingly in 90 per cent. alcohol, and it is identical with Prior's achroodextrin III⁴ and with Ling and Baker's maltodextrin- β .⁵

¹ I am able to confirm this statement and have long arrived at the same conclusion.—A. R. L.

² *Journ. Chem. Soc., Trans.*, 1899, 75, 292.

³ The constants observed by various investigators for this compound are as follows: Brown and Morris (*Journ. Chem. Soc., Trans.*, 1885, 563) $[\alpha]_{D^{38}} 193.1$ $\kappa_{D^{38}} 21.1$; equal to according to Brown and Millar (*ibid.* 1899, 287), $[\alpha]_D 180^{\circ}$, R. 34.5; Lintner and Düll (*Zeit. ges. Brauer.*, 1894, 339), $[\alpha]_D 183^{\circ}$, R. 34.5; Ling and Baker (*Journ. Chem. Soc., Trans.*, 1897, 71, 517); $[\alpha]_D 180^{\circ}$, R. 32.8.

⁴ *Bayerisches Brauer. Journ.*, 1896, 6, 385.

⁵ The constants observed by Prior were $[\alpha]_D 171.1^{\circ}$, R 42.5; whilst Ling and Baker found with their preparations $[\alpha]_D 171.6^{\circ}$, R 43.0.

Both final dextrin II and γ -maltodextrin yield a mixture of maltose and isomaltose (see below) on complete hydrolysis with malt-extract. The presence of an "isomaltose" residue in the γ -maltodextrin molecule, as well as in that of the parent substance, final dextrin II, explains the fact that final dextrin II yields more apparent maltose than γ -maltodextrin when both are hydrolysed by malt-extract under the same conditions.

"*Isomaltose*", or, as Syniewski prefers to term it, *dextrinose*, $C_{12}H_{22}O_{11}$, is obtained in a pure state by hydrolysing final dextrin I, $C_{73}H_{124}O_{62.3}$. The product contains maltose besides dextrinose, the latter being separated by its more sparing solubility in ethylic alcohol. He did not succeed in crystallising dextrinose; but he found that a preparation dried over phosphoric anhydride in a vacuum had an elementary composition and molecular weight corresponding with the formula $C_{12}H_{22}O_{11}$. It melted with strong frothing at $82-83^{\circ}C$. Its constants were $[\alpha]_D 141.4^{\circ}$ at $20^{\circ}C$., and $R 84.5$. The phenyl-osazone melts at $152-153^{\circ}C$. Dextrinose is not identical with the isomaltose of E. Fischer as it ferments with yeast. It will thus be seen that Syniewski's dextrinose closely resembles in every respect the "isomaltose" which Lintner claims to have isolated from the products of the action of diastase on starch paste (see p. 164). It is also noteworthy that Syniewski confirms the observation of Ling and Baker that it is of the nature of a dextrin of low molecular weight.

The Carbinol Hydrolysis of Starch.—Amylodextrin $(C_{54}H_{96}O_{48})_n - \frac{3n}{2} H_2O$, is obtained by heating starch paste in an autoclave for twelve hours at $140^{\circ}C$. When the perfectly dry substance is mixed with anhydrous soda and an excess of acetic anhydride and the mixture gently boiled for two hours, an acetyl compound, identical with that prepared from starch by Schützenberger and Naudin,¹ is obtained. It is a white amorphous powder melting at $280-281^{\circ}C$. Analysis showed that this acetyl derivative had the formula

$$n[C_{54}H_{66}O_{48} - \frac{3}{2} H_2O(C_2H_3O)_{30}].$$

When a 10 per cent. solution of amylodextrin is allowed to stand for several days sphaero-granules of a reversion product insoluble in water separate out. Unlike starch in the form of granules, this product is rapidly dissolved and hydrolysed by malt-extract in the cold. The starch molecule, the amylogen complex of which is assumed to contain twenty-seven free hydroxyl groups, is practically not attacked by malt-extract in the cold; it must first undergo a carbinol hydrolysis. The amylogen complex of amylodextrin contains thirty free hydroxyl groups, and this dextrin is readily hydrolysed by malt-extract yielding the whole of the potential maltose. The above-mentioned reversion product of amylodextrin contains two more hydroxyl groups than starch, and although it is readily hydrolysed by malt-extract, it only yields two-thirds of the potential maltose.

Syniewski criticises the theories of Lintner and Düll and of Brown and Millar on the mechanism of the hydrolysis of starch by diastase and discards

¹ *Annalen*, 1902, 160, 77.

both of them as untenable. He regards the starch molecule as made up of amylogen residues (see p. 81); and he assumes that the nine glucose groups which make up the amylogen residue, $C_{54}H_{90}O_{45}$, in the starch molecule are connected together by nine carbonyl bonds of three different kinds, which he denotes as α -, β -, and γ -bonds. The amylogen residue is depicted thus:

The three monocarbonyl bonds which unite the three maltose residues with the residue of final dextrin I are α -bonds. The three monocarbonyl bonds which are between the three glucose groups of final dextrin I are β -bonds. The bonds which unite the glucose groups of the maltose residues are γ -bonds. The amylogen residue may also be represented as follows:

The composition of starch is represented by the formula

whilst that of amylopectin is represented by the formula $n [C_{54}H_{90}O_{45} + \frac{3}{2} H_2O]$.

By the action of malt-extract heated at $78^\circ C$., amylopectin may be completely converted into final dextrin II, $C_{36}H_{52}O_{31}$. From this it follows that n must be a whole number. Final dextrin II is formed from two different amylogen residues, and this same conclusion is arrived at from the assumption that the β -carbonyl bonds are disrupted in its formation. From certain geometrical considerations, Syniewski concludes that the value of n in the potato-starch molecule is 4. This leads to the molecular formula $C_{216}H_{360}O_{180}$, which is graphically represented on p. 81.

According to this formula, each amylogen residue in the starch molecule is united by six carbinol anhydride bonds. Three of these bonds are between final dextrin I-residues and three between maltose residues. The former are denoted d -bonds, the latter m -bonds. The single lines between the C_{18} -groups forming a dextrin ring are β -carbonyl bonds; the double lines uniting two adjacent C_{18} -groups are carbinol bonds, one being a d -bond and the other an m -bond.

It is conceivable that the molecular constitutions of other starches are analogous, but contain a different number of amylogen residues, thus 2, 8, or 20.

By heating starch paste in an autoclave at $140^\circ C$., the hydrolysis of all the m -bonds takes place and six molecules of water are added, the resulting compound, amylopectin, having the formula $C_{216}H_{372}O_{186}$.

Amylodextrin is hydrolysed by unheated freshly-prepared malt-extract at the ordinary temperature, so that in each amylogen residue one β -carbonyl bond and all the α -carbonyl bonds are broken, maltose and final dextrin I being produced. There are also formed other intermediate compounds which give colorations with iodine; these are the amylo-dextrins and the erythro-dextrins of other authors. Syniewski denotes them α -maltodextrins. Malt-extract then acts on the remaining β -carbonyl bond of the final dextrin I, whereby dextrinose results, and finally the δ -carbonyl bond in dextrinose is disrupted and glucose is formed.

Malt-extract which has been heated to 78°C . for fifteen minutes acts at the ordinary temperature principally on the β -carbonyl bonds, the resulting products consisting of two C_{18} -residues united by δ -carbonyl bonds. This is final dextrin II, which may be hydrolysed by freshly prepared, unheated malt-extract at the α -carbonyl bonds, giving rise to γ -maltodextrin; finally, the second maltose residue is split off and dextrinose remains, which, by the prolonged action of malt-extract, yields glucose.

In practice starch is saccharified at temperatures of 65 – 70°C ., and without doubt α - and β -carbonyl hydrolytic products of amylo-dextrin and also α -hydrolytic products of final dextrin II are formed. Even when the process is not carried to its final point, the maltodextrins of Brown and Morris and of Ling and Baker, the achroodextrins of Lintner and of Prior, and the isomaltose of Lintner, and even glucose are formed.

In order to differentiate between the two typical products of starch, sugar, and dextrin, Syniewski defines a sugar as a compound in which the glucose residues are united by carbonyl bonds only; the highest sugar in the series is amylogen, and the lowest glucose. A dextrin, on the other hand, contains at least one carbonyl bond; the highest dextrin in the series is amylo-dextrin, and the lowest dextrinose.

Ling and Davis on the Action of Malt Diastase on Potato-starch Paste.—These authors showed¹ that when malt diastase is heated in aqueous solution above the temperature at which the activity of the enzyme is at its optimum, namely 55°C ., the reaction with potato-starch paste at about 55°C . is not only slower, but different products are formed; thus δ -glucose can be readily isolated from them after the reaction has been allowed to proceed for several hours. Special experiments, employing the same quantities of diastase which had not been heated in solution above 55°C ., showed that δ -glucose is not formed either from starch paste or from maltose. It therefore appears that the production of this sugar is connected with the previous heating of the hydrolytic agent in solution above 55°C . As a result of a very large number of more recent experiments,² the authors arrived at the following conclusions: The effect of heating a solution of diastase as above indicated is to weaken its action and also to produce an alteration in the enzyme molecule, which is moreover a permanent one, for the diastase retains

¹ *Journ. Fed. Inst. Brew.*, 1902, **8**, 475.

² *British Assoc. Report, Southport*, 1903; *Journ. Chem. Soc., Trans.*, 1904, **85**, 16–29.

its altered properties when reprecipitated from its solution by alcohol and allowed to act on starch paste at or below 55°C . The alteration of the diastase is assumed by the production of *d*-glucose when it acts on starch paste, and it appears to commence when a solution of the enzyme is heated below 60° ; although, judging from the small amount of *d*-glucose formed by its action, the change at the last-named temperature is not complete.¹ As the temperature to which the solution is heated prior to allowing it to act on the starch paste is increased, the amount of *d*-glucose it is capable of producing also increases, the maximum amount being obtained by the action of diastase which has been so heated in solution at $68\text{--}70^{\circ}\text{C}$. for fifteen to thirty minutes. Above this temperature the destruction of the enzyme is so rapid that a much larger proportion of it has to be employed to attain the stage of the reaction at which *d*-glucose appears. Still *d*-glucose is formed by diastase which has been restricted at temperatures up to 78°C ., and probably above this. It has been observed in all cases that when, after the maximum amount of *d*-glucose has been formed, the solution is kept at the temperature of hydrolysis, usually 55°C ., the sugar just mentioned diminishes in amount, and the occurrence of this apparent condensing action of the enzyme may probably explain the failure in several cases to detect *d*-glucose among the products of hydrolysis (compare paragraph on p. 183). The maximum amount of *d*-glucose formed in any case does not exceed about 12 per cent. of the total hydrolytic products.

E. Prior² has confirmed the observation that glucose is produced by the action on starch of diastase solution which has been previously heated. He believes this to be explained by the assumption that two enzymes are present in diastase.

Ling and Rendle³ find that commercial concentrated malt-extracts invariably contain glucose, and they believe that this glucose is produced during the evaporation of the extract, glucose being always produced by the action of diastase on any of the products of the partial hydrolysis of starch by diastase as stated in the following paragraph.

In a paper read before the British Association in 1903,⁴ Ling pointed out that Brown and Millar have shown that the so-called stable dextrin—one of the products of the hydrolysis of potato-starch paste by diastase—is converted by the further action of diastase into a mixture of about equal parts of *d*-glucose and maltose. The observation of Ling and Davis that no *d*-glucose is formed when unrestricted diastase acts on starch paste, stands in apparent antithesis to this. Ling confirmed the statement of Brown and Millar, and found further that other isolated products of partial diastatic action yield a proportion of *d*-glucose when submitted to the further action of unrestricted diastase; thus the maltodextrin- α of Ling and Baker, when treated in 3 per cent. solution with an active preparation of diastase at 55°C . for 140

¹ It should be observed that this production of *d*-glucose is not due to a maltase, as the heated diastase solution does not convert maltose into glucose.

² *Wöchensch. für Brauerei*, 1904, **21**, 349.

³ *Analyst*, 1904.

⁴ *British Association Report, Southampton*, 1903; *Journ. Fed. Inst. Brew.*, 1903, **9**, 446.

hours, gave the constants $[\alpha]_{D_3.93}$ 127.6°, $R_{3.93}$ 105.6, corresponding approximately with maltose 90 per cent., *d*-glucose 10 per cent. The presence of 10.5 per cent. of *d*-glucose in the product was proved by weighing the phenylglucosazone formed under standard conditions. Taking into account the fact that potato-starch paste is never completely converted into maltose, although the final product has the constants of that sugar, and that a substance is always present of the nature of the isomaltose of C. J. Lintner, the simple dextrin of Ling and Baker, and the dextrinose of Syniewski, which when isolated and submitted to the action of diastase yields *d*-glucose, Ling suggested that the reason no *d*-glucose can be detected among the products of the action of unrestricted diastase on starch paste is that that sugar is immediately condensed by the action of the enzyme, dextrinose being formed. When, however, the diastase is previously heated, its condensing action is weakened, and the *d*-glucose formed can be isolated. Attempts to condense *d*-glucose or mixtures of it with maltose were not successful.

Action of Diastase on the Starch Granules of Raw and Malted Barley.—Ling¹ states that the whole of the published data referring to the hydrolysis of starch by diastase have been derived from the study of the action of the enzyme on potato-starch paste, and the application of these to practical purposes has led to misleading and erroneous conclusions. The starches of barley and other cereals differ from that of the potato in being readily attacked by a solution of diastase in the ungelatinised condition. He carried out a series of mashes with barley and malt starch of various origin, the starch being mixed with the diastase preparation in the dry state and mashed with water at different temperatures for two hours.

The following table illustrates the results obtained :

Starch employed.	Mashing Temperature.	$[\alpha]_{D_3.93}$	$R_{3.93}$
Barley 1899 sample	60°	140.8°	88.7
Barley 1899 sample	65.5°	143.4°	88.2
Barley 1899 sample	71°	145.1°	80.6
Barley 1902 sample	60°	150.7°	84.2
Barley 1902 sample	65.5°	152.3°	79.0
Barley 1902 sample	71°	163.8°	77.8
Kilned malt	60°	150.9°	84.4
Kilned malt sample	65.5°	156.1°	80.6
Kilned malt sample	71°	161.3°	67.2
Low-dried malt	60°	151.6°	85.3
Low-dried malt sample . . .	65.5°	152.5°	83.3
Low-dried malt sample . . .	71°	165.7°	66.6
Barley-starch (Paste) . . .	65.5°	168.6°	52.4
Potato-starch (Paste) . . .	60°	154.5°	81.8
Potato-starch (Paste) . . .	65.5°	155.6°	71.5
Potato-starch (Paste) . . .	71°	167.1°	55.3

The very great differences between the constants yielded by the starch mashes and the starch-paste conversions are apparent. It is also to be noted

¹ *British Association Report, Southport, 1903; Journ. Fed. Inst. Brewing, 1903, 9, 446.*

that the starches from different barleys give different constants. Ling brought forward evidence showing that in the process of mashing, as conducted in breweries, the starch granules are dissolved directly by the diastase and are not gelatinised prior to hydrolysis, as it is usually stated they are. It is probable that the products formed in these starch mashes are different from those resulting from the hydrolysis of starch paste.

Accelerating Compounds.—Effront has shown¹ that asparagine, dicalcium phosphate, aluminium acetate, ammonia or potash alum, as well as vanadium salts and albuminoid substances exert a favourable effect on the saccharification of starch by diastase. This only appears to hold, however, when the quantity of diastase present is small. With an excess of diastase the presence of either of these substances has no effect on the saccharification. Again, even with a very small quantity of diastase the addition of any of the substances above named does not affect the ultimate degree of saccharification provided sufficient time be allowed; their influence must therefore be regarded as merely acceleratory. Ford² brings forward experimental evidence showing that diastatic action attains its maximum in neutral solution, using the term "neutral" in the sense that water is neutral. He denies that asparagine and the other substances mentioned above augment diastatic action unless there has been a previous restriction due to the presence of traces of alkali, copper, &c., in the starch. The restricting influence of acids depends on their dissociation,³ the greater the amount of free hydrogen ions the greater the restriction, although it is possible the anion of certain acids may have some influence. Effront⁴ in reply to Ford brings forward fresh evidence in support of his previous statements. The accelerating action of asparagine is independent of the alkalinity of the medium. Succinamide is shown to retard diastatic action. Sarcosine, alanine, leucine, glutaminic acid, hippuric acid, creatine, creatinine and the products of the action of peptase or of tryptase on the albuminoids favour diastatic action, and Effront concludes that this effect is due to the presence of the amino group in these compounds. Ford and Guthrie⁵ in a rejoinder urge that the preparations of starch employed by Effront were impure and alkaline by his own showing. They bring forward additional experimental evidence, which leads them to the following conclusions: Asparagine and the other amino acids mentioned above have no specific influence in augmenting the action of diastase; the apparent augmentation of action sometimes obtained by the addition of these amphoteric substances (or of feeble acids) is due to their neutralising alkaline or other impurity in the starch or enzyme solution. Normal amylolytic action takes place in neutral solution. In the plant, this neutrality is brought about by equilibrium between the basic and acid products present. They point out that purified potato-starch has the properties of a weak acid; it is capable of yielding negative ions under the influence of strongly positive ones.

¹ *Les Enzymes*, Paris, 1899.

² *Journ. Soc. Chem. Ind.*, 1904, 414.

³ Compare Ling, *Journ. Fed. Inst. Brewing*, 1902, 8, 446.

⁴ *Monit. Scient.*, 1904, 61, 561.

⁵ *Journ. Chem. Soc., Trans.*, 1906, 89, 76.

Coagulation of Starch.—Fernbach and Wolff¹ have pointed out that extracts of green barley, green wheat and green oats as well as the leaves of clover contain, besides a saccharifying enzyme, another of these bodies which precipitates or coagulates starch solutions. It is suggested that solid starch is precipitated in the milky juices of these cereals by the agency of this enzyme, to which they give the name "amylocoagulase." The enzyme is contained in malt, even in kilned malt, since although it is destroyed when an aqueous solution is heated for five minutes to 63° C., it resists high temperatures in the dry state. In malt therefore two antagonistic enzymes occur, diastase and coagulase, and if the former be allowed to predominate no coagulation may occur in starch solutions. High temperatures and dilute starch solutions favour diastatic action, but if these be allowed for, the first signs of coagulation appear earlier the greater the quantity of malt-extract employed. Coagulation proceeds more readily the lower the temperature at which the starch has been rendered soluble (starch solutions prepared by heating with water under pressure are here referred to) and the shorter the duration of the heating process. When coagulation has proceeded rapidly, it first appears as a turbidity and subsequently clots are formed; slow coagulation is characterised by the deposition of a white powdery precipitate. With a 2 per cent. solution of starch coagulation takes place at 8° C., but not at 22° C., whilst with a 4 per cent. solution coagulations are readily produced at 15–25° C.; lower temperatures are, however, always more favourable. Addition of 0.080 gram of caustic soda per litre to a solution of starch assists coagulation by restraining the action of diastase, as coagulase is less sensitive to the action of alkalis than diastase. A portion of the coagulated matter consists of amylocellulose. Maquenne, Fernbach, and Wolff² state that if the coagulum is examined immediately after its formation, very little amylocellulose is detected, but the amount increases and may finally constitute as much as one-half the total coagulated matter.

Conversion of Amylocellulose into Starch.—According to E. Roux³ amylocellulose (see also p. 75) may be prepared by treating an 8 per cent. potato-starch paste which has previously been kept at a low temperature for about a week with an excess of malt-extract at 56° C. The product is washed with water at 120° C. and dried on a plate. When heated with water at 155° C. it is converted into a thin solution of starch, and when the heating is protracted spherical granules identical with those of natural starch separate (see also p. 75).

Proteolytic Enzymes and their Action.—Two proteolytic enzymes, pepsin (or peptase) and trypsin (or tryptase), can be readily obtained from the stomach and pancreas of animals. Gorup-Besanez succeeded in extracting enzymes of this nature from tares, hempseed, linseed, and germinated barley; but his work was for some years considered doubtful, since the method he employed for detecting the products of their action is open to criticism. A

¹ *Journ. Inst. Brewing*, 1904, **10**, 216.

² *Compt. rend. de l'Acad. des Sciences*, 1904, **138**, 49.

³ *Ibid.* 1905, **140**, 440.

powerful enzyme of this nature has been isolated from the latex of the tropical plant *Curica Papaya*. It digests proteïds with great facility, converting them into proteoses and peptones, a small quantity of amides being simultaneously formed. Certain plants, which possess organs peculiarly arranged for entrapping insects, are also provided with a powerful proteolytic enzyme, by means of which the insects so caught are digested and utilised in the nutrition of the plant. The fluid contained in the pitchers of a genus of insectivorous plants, the *Nepenthes*, when slightly acidified, instantaneously dissolves coagulated egg albumin.

Action of Pepsin on Proteïds.—The action of this enzyme can be readily studied on the proteïd, egg albumin, which is a substance easily obtained in a state of comparative purity. It is only necessary to boil an egg until its contents are completely hard, remove the white from the yolk, and cut the former into thin slices, or finely divide it by rubbing through a sieve. The finely divided albumin is then placed in a very dilute solution of pepsin which contains 0.2 per cent. of hydrochloric acid, and kept at a temperature of 38° C. (100° F.) for two or three hours, when the bulk of the albumin will have by this time dissolved. If a little of the solution is now taken out, filtered, and exactly neutralised with an alkali, a precipitate falls. This consists of syntonin or acid albumin, and represents the first stage of the action of pepsin on a proteïd. If the original mixture is allowed to stand at the temperature before indicated for several hours longer, a portion of the solution removed, and, as before, exactly neutralised with an alkali, a much smaller precipitate will form. The bulk of the syntonin has by this time been converted into a group of bodies collectively called “proteoses,” together with a little peptone. The presence of the proteoses may be detected by removing the syntonin from the neutralised liquid by filtration, and saturating the filtrate with ammonium sulphate. The heavy precipitate which is thrown down consists of the proteoses. Nitric acid also precipitates these bodies from a fairly strong solution as white flocks, which dissolve when the mixture is heated and reappear as it cools. The group of the proteoses may be separated into its three members, the proto-proteoses, the hetero-proteoses, and the deutero-proteoses, in the following manner: The liquid which contains all these three members is saturated with sodium chloride; this precipitates the proto-proteose and hetero-proteose. The latter of these two bodies is insoluble in pure water; the precipitate obtained above, which contains these two bodies, is therefore dissolved in a dilute solution of sodium chloride and dialysed. When the salt has been completely removed by dialysis, the hetero-proteoses are precipitated, and the proto-proteoses remain in solution. In order to obtain hetero-proteose in considerable quantity, the digestion of the original mixture is allowed to continue for some time longer, when the transformation of a large portion of the proto-proteoses and hetero-proteoses is carried a stage further, and they are converted into deutero-proteoses and peptones. In order to obtain the deutero-proteoses, the solution is first saturated with sodium chloride, then acetic acid added drop

by drop until a precipitate ceases to be formed. The proto- and hetero-proteoses are not completely removed from a solution by saturation with salt, it is only after the addition of acetic acid that the last portions are precipitated ; these carry down with them a little deuteroproteose. The deuteroproteose can then be obtained by dialysing the acid salt solution until it is quite free from sodium chloride, evaporating to small bulk, saturating with ammonium sulphate, and filtering off the precipitate. This precipitate consists of the deuteroproteoses ; the peptones remain in solution. The precipitate thus obtained is then dissolved in a little water, the solution dialysed, evaporated to small bulk, and poured into strong alcohol ; this reprecipitates the deuteroproteoses, which are then washed with alcohol and dried over sulphuric acid. If the digestion of the original mixture is carried on for a much longer period, say several weeks, a large quantity of the original albumin will have been converted into peptones. In order to obtain these bodies, the solution, after concentration, is saturated with ammonium sulphate, which throws down all proteïds out of the solution with the exception of the peptones. In order to obtain the peptones from the saturated ammonium sulphate solution, the precipitated proteoses are filtered off, and the filtrate concentrated so as to remove the bulk of the salt by crystallisation. The solution is then boiled with barium carbonate to remove the remainder of the ammonium sulphate ; the ammonia volatilising, and the sulphuric acid being precipitated as barium sulphate. The boiling is continued until the liquid ceases to evolve ammonia ; it is then filtered, concentrated to a syrupy consistence, and poured into strong alcohol. Peptone thus prepared is a gummy substance, which on dehydration with alcohol is obtained as a yellowish powder of a very hygroscopic nature and having a bitter taste. When perfectly dry it dissolves in water with the evolution of heat. From a large number of experiments, which we cannot enter into here, it has been shown that the proteïd molecule consists of two distinct groups or radicals, from which the various products of proteolytic action are derived. The following schematic arrangement will, without going into further details, give a general idea of the series of changes :

The great distinction between these two groups, which are called the “hemi- and anti-groups,” and which appear to exist in about equal quantities, is that the peptone of the former can be further broken down by the action of trypsin into a number of amide bodies, whilst the peptone of the latter cannot be so broken down. In every digestion a variable quantity of a substance is left undissolved, which is called “anti-albumid” ; it is extremely resistant to the further action of the proteolytic enzymes, but, by treatment

with strong solution of pepsin and acid, may be partially converted into anti-deutero-albumose, and finally into anti-peptone.

Action of Trypsin on Proteïds.—Trypsin, which is obtained from the animal pancreas, is a much more powerful proteolytic agent than pepsin, and capable of carrying on the breaking-down process to a much greater extent. As a consequence of this, the products of trypsin proteolysis are much more complicated than those of pepsin proteolysis. Trypsin can act in an alkaline, neutral, or slightly acid medium, but its action is most energetic in an alkaline medium. It occurs in the pancreatic juice, which is a slightly alkaline liquid. When caused to act upon a proteïd in a solution containing from 0.5 to 1.0 per cent. of sodium carbonate, the stage of the proto- and hetero-proteoses is passed through so rapidly that it can seldom be observed, and the proteïd matter is quickly converted into deutero-proteoses. These are rapidly transformed into hemi-peptone and anti-peptone in nearly equal quantities. The hemi half is now quickly broken down into a number of amide bodies, such as leucine, tyrosine, &c., whilst the remaining half suffers no further cleavage. The great peculiarity with regard to trypsin is its power to break down the hemi portion of the proteïd molecule into a number of crystalline bodies. These are leucine or amino-caproic acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$; tyrosine or hydroxy-phenylaminopropionic acid, aspartic and glutamic acids. These bodies have long been known to be products of trypsin proteolysis, but more recently two other bases have been discovered, lysine or diamino-caproic acid, $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$, and lysatine, $\text{C}_6\text{H}_{13}\text{N}_3\text{O}_2$, the constitution of which is not yet known. Small quantities of ammonia have also been detected amongst the products of the action of trypsin on the proteïds.

Proteolysis during the Germination of Barley.—It is quite certain that proteolysis goes on to a marked extent during the germination of barley, since a portion of the insoluble proteïds are converted into soluble proteoses, and amide bodies are formed as well. The vegetable proteolytic enzyme or enzymes resemble trypsin in their action when we regard the profound changes which are brought about in the proteïd molecule under their influence.

Proteolysis of the Proteïds a Necessity.—In the germination of the barley, since the proteïd matter of the grain has to travel from the endosperm to the germ, it is necessary that those proteïds which are in an insoluble condition should be rendered both soluble and diffusible, because, in passing from cell to cell, they have to traverse the cell-walls. The wort which the brewer produces must also contain proteïd bodies of a nature readily assimilable by yeast, or that organism would speedily become so weakened as to be incapable of fulfilling its functions properly. The diffusible and assimilable bodies requisite for this purpose are produced by the proteolysis of the proteïds originally contained in the barley, effected for the most part during its germination, afterwards under certain conditions to a slight extent during the mashing process. The albumin and globulins which occur in the barleycorn seem completely indiffusible, the proteoses are very slightly diffusible, whilst the

peptones have very considerable powers of diffusion, and the amides are highly diffusible bodies.

Proteolytic Enzymes of Malt.—The question whether raw or malted grain contains a proteolytic enzyme, although a most important one from a practical point of view, has only been definitely decided in the affirmative quite recently. In 1874 it was shown by Gorup-Besanez¹ (p. 185) that germinating barley contained an enzyme capable of dissolving animal fibrin. Neumeister² observed that the products of this action exhibited the reactions of albumose and peptones. Laszczynski³ was unable to confirm the experiments of Gorup-Besanez and of Neumeister, and so arrived at negative conclusions with regard to the existence of a proteolytic enzyme. Loe⁴ arrived at similar conclusions.

Shortly after the publication of the researches just referred to, Fernbach and Hubert⁵ demonstrated the existence of the proteolytic enzyme in several ways. Petit and Labourasse⁶ made some interesting observations on the auto-digestion of malt-extracts, plainly demonstrating the presence of a proteolytic enzyme in malt. Windisch and Schellhorn,⁷ who were perhaps the first to place our knowledge of the proteolytic enzyme on a really satisfactory basis, showed that the enzyme exists as a free agent in malt and not as a zymogen; that its activity is greatest in a dilute alkaline solution, that is to say, it possesses tryptic properties; that dilute acid also increases its activity; and that raw barley (except when it has been badly weathered) does not contain the enzyme. Using 20 c.c. of an 8 per cent. gelatin-thymol (containing 0.5 per cent. of thymol) and 3 grains of ground or crushed grain, these authors found that all barleys, after a 24 hours' steep and a 4 days' flooring, caused the liquefaction of gelatin when incubated at 40° C. for 24 hours. They suggest as a test for weathered barleys that 3 grains of the ground barley should be added to 20 c.c. of a 6 per cent. gelatin-thymol, and the mixture incubated for 10 days. They found that the optimum temperature of the enzyme is 50° C.; that small quantities of mineral and organic acids and of acid salts increase proteolytic activity (not, however, the gelatin liquefying power); and that when malt or barley extracts are mixed, the proteïds of the barley are degraded. By various modifications of the method of Gorup-Besanez, Windisch and Schellhorn succeeded in precipitating the enzyme; the preparations obtained were, however, much weakened in power.

Weiss⁸ carried out a long series of experiments on this subject and arrived at the following conclusions: An aqueous extract of green malt possesses pronounced proteolytic properties, which can be demonstrated by auto-digestion as well as by allowing it to act on albuminoid matters of

¹ *Ber. d. Deutsch. chem. Ges.*, 1874, **7**, 1478.

² *Zeit. Biol.*, **30**, 477.

³ *Zeit. ges. Brauw.*, 1899, **22**, 71, 83, 123 and 140.

⁴ *Ibid.* 1899, **22**, 212.

⁵ *Compt. rend. de l'Acad. des Sciences*, 1900, **130**, 1783; **131**, 293.

⁶ *Ibid.* 1900, **131**, 349 and 394.

⁷ *Wochensch. Brauw.*, 1900, **17**, 334.

⁸ *Compt. rend. Trav. Laborat. Carlsberg*, 1903, **5**, 135.

other origin. By precipitations with stannous chloride and tannin two different phases in the proteolysis can be shown to exist—a hydrolytic or peptic phase in which albumoses are formed, and a tryptic phase in which crystalline non-proteid compounds are formed. It must be assumed, therefore, that two enzymes are present, a peptase and a tryptase. The peptic action proceeds with rapidity and quickly attains its final stage, whereas the tryptic action is slow and continues until all the peptic products have been decomposed. The influence of temperature, quantity of malt-extract, concentration of the solution of proteid, and duration of the experiment, depends on the variation of other factors. The tryptic action appears not to occur, or only to a very feeble extent, in a neutral medium. The addition of a very small amount of acid accelerates the action considerably, whilst that of alkali exerts a retarding influence.

The influence of acids and alkalis is explained, according to the theory of Fernbach and Hubert, by the presence of primary and secondary phosphates in the malt-extract, the former having a favourable, the latter a retarding, effect on the proteolysis. The tryptic phase is retarded by alcohol according to the quantity of the latter added; it is also retarded by thymol, chloroform, formaldehyde, benzoic acid, and salicylic acid. The peptic action is less affected by formaldehyde. Neither the peptic nor the tryptic action is sensibly influenced by toluene, which accordingly is a good preservative of the proteolytic power of malt-extract. The view that at least two enzymes are present is supported by the fact that an enzyme preparation precipitated by alcohol is almost devoid of tryptic properties, whilst the peptic power is retained. The enzymes are equally soluble in water, dilute lactic acid, and glycerol. They are slightly diffusible through an animal membrane. They may be slowly heated to 95° C. in the dry state; but they are destroyed at 70° C. in presence of water. Freezing an extract of malt does not destroy its tryptic power, and it must be assumed that the same is true with regard to its peptic power. Light has a retarding influence (if any) on both the enzymes. Weiss found that an extract of raw barley has only a very feeble peptic power, and is quite devoid of tryptic power. The proteolytic action becomes first plainly evident on the third day of germination; it attains its maximum by the sixth day. Raw barley appears to contain proenzymes (zymogens), which can be rendered active by treatment with dilute lactic acid at an appropriate temperature.

In a subsequent paper Weiss¹ described a continuation of his studies. He asserts that the enzymes of green malt comprise a milk-coagulating enzyme similar to rennet and the two proteolytic enzymes above referred to. He considers that the proportion of nitrogenous matter, which is converted into a form in which it is not precipitable by stannous chloride, is a measure of the activity of the peptase, whilst the proportion which is converted into a form unprecipitable by tannic acid affords a measure of the action of the tryptase.

The behaviour of these two enzymes differs according to temperature.

¹ *Zeit. ges. Brauw.*, 1904, **27**, 385.

Experiments made with a 2 per cent. solution of wheat-glutin in presence of 0.4 per cent. of lactic acid showed the first phase of proteolysis corresponding to the action of the peptase (*i.e.*, degradation down to albumoses) takes place to a considerable extent at relatively low temperatures, with a distinct maximum at 51° C., at which temperature the action is nearly twice as rapid as at 35° C. and at 60° C. The highest temperature at which the peptic proteolysis takes place is about 70° C. The more profound transformation attributed to the tryptase (degradation of the albumins down to amides, and products unprecipitable by tannic acid) is decidedly slower than the first phase. The optimum temperature lies between 45° and 50° C.; at temperatures below 35° C. the action is very slight, and it ceases at about 70° C. (138° F.). In brewery practice, the result of the activity of the proteolytic enzymes will be different, according to whether the mash is made up at 35° C. (95° F.) and allowed to stand at that temperature for a certain time or whether the mash is started at 50–60° C. (122–140° F.); if the mash be started at 60–63° C. (140–145° F.) proteolytic action will be practically nil. Free alkalinity and excessive acidity are unfavourable to both kinds of proteolytic action; alcohol, and most antiseptics, interfere with the activity of the tryptase.

The proteolytic enzymes of malt will attack albumin of any origin, whether vegetable or animal, but in different degrees, so that the albumoses of wort can be increased, if desired, by the addition of foreign albumins. The peptase will withstand the operation of precipitation by alcohol, but the tryptase will not.

There is little doubt that the germinating power of barley depends, to a large extent, on the proteolytic enzymes of the grain, and the determination of these should play a part in judging the malting value of barley.

The proteolytic enzymes are not affected by kilning, and they exert a distinct influence on the quality and quantity of the nitrogenous matters of the wort, according to the method and temperature of mashing adopted. In mashing experiments, started at 35° C. (95° F.), with a slow rise, it was found that no appreciable increase of the albumoses of the wort took place; the increase of the total nitrogen of the wort, which occurred chiefly in the second hour, was due mainly to an increase of the lowest forms (amides, &c.), and these continued to increase, owing to the action of the tryptase on the albumoses at the moment of their formation by the peptase.

A considerable quantity of soluble albumin survived the mashing process, and was only removed by coagulation in the copper. Hence, it is concluded that malt contains certain forms of albumin which are not attacked by the proteolytic enzymes. It may be assumed that the peptase, which acts rapidly, and at low temperatures, will have converted all the albumin, which is susceptible to its action, during the malting process, and that most of the proteolysis performed in the mash tun is due to the tryptase. The method of brewing with low initial temperature, and with a slow rise, is not favourable to a high albumose-content of the wort. This object would be better attained, for instance, by Windisch's abbreviated high-temperature mashing process.

The albumose-content of the wort might also be raised by the addition of raw grain meal of a nitrogenous character to the mash tun. Weiss investigated this point by the addition of wheat flour; the results showed an increase of the soluble albumins and albumoses, and a decrease of the amides of the wort, the total nitrogen being the same as in an all-malt wort. But wheat flour is not available in practical brewing, because it causes difficulties in clearing and want of stability. The addition of maize flour to the mash tun caused, on the other hand, a decrease of total nitrogen, soluble albumin, and albumoses, whilst increasing the relative proportion of amides. Hence, the use of maize flour would tend to make the beer as poor as possible in nitrogen, without decreasing the nutrition of the yeast.

In connection with this subject two important papers by P. Schidrowitz¹ must be referred to. This author describes a method of determining the relative proteolytic power of malt.

The Nitrogenous Constituents of Malt.—These are partly of a proteid and partly of an amide nature, and though they have often formed the theme of investigation, as yet no thoroughly reliable process has been discovered for their separation and quantitative estimation. The usual plan has been to extract the malt with water, and boil the aqueous solution after the addition of a little cupric hydroxide or ferric acetate. The precipitate thus formed was filtered off and considered to be albumin. It contained, however, part of the proteoses as well. The filtrate was then acidified with hydrochloric acid and solution of phosphotungstic acid added, or tannin without previous addition of acid, and the precipitate thus obtained was called "peptone." It consisted, however, mainly of proteoses, very little, if any, true peptone being found in malt. The remainder of the nitrogenous matter left in solution was assumed to represent the amides. W. J. Sykes² has proposed the following process for the estimation of the proteids in malt: The malt is extracted with cold water, filtered, and the albumin in the filtrate coagulated at a temperature of 60° C. (140° F.). The coagulum is filtered off and weighed. The filtrate is then boiled for a short time, when a second precipitate forms, which is filtered off and weighed. This was considered to represent the globulin, but from the recent experiments of Osborne it is extremely doubtful if all the globulin can be separated by boiling. The filtrate is then evaporated to small bulk and saturated with ammonium sulphate, when a precipitate consisting of the proteoses (and probably some globulin) comes down. This is filtered off, washed with saturated solution of ammonium sulphate, dried and weighed, the weight of the ammonium sulphate adhering to the precipitate and filter-paper being determined and deducted. The filtrate from this precipitate is then diluted with an equal quantity of water and solution of tannic acid added. This would precipitate peptone, if it were present; but no precipitate of any moment was ever observed, and this led to the conclusion that real peptone does not occur in malt. Szymanski³

¹ *Journ. Fed. Inst. Brewing*, 1903, **9**, 361; *Journ. Inst. Brewing*, 1904, **10**, 166.

² *Trans. Inst. of Brewing*, **4**, 173.

³ *Landwirthsch. Vers.-Station*, **32**, 389.

considered that he had separated peptone from malt, but from the process he adopted for its isolation, the substance which he obtained and mistook for real peptone was evidently deuto-proteose.

W. J. Sykes, in experimenting on the proteids of malt, was never able to detect a hetero-proteose on dialysing the precipitate thrown down by ammonium sulphate. A precipitate was always yielded on saturating malt-extract with sodium chloride and adding a little acetic acid, and the body is left in solution which Szymanski mistook for peptone. In all probability, therefore, the two proteoses are proto- and deuto-proteose. (See also proteids of malt, p. 106.)

Bungener and Fries have given the following analysis of the soluble nitrogenous constituents of a sample of barley and of the malt made from it :

	Barley. Per cent.	Malt. Per cent.
Total nitrogen	1.690	1.580
Nitrogen as albumin	0.161	0.230
Nitrogen as peptone	0.040	0.060
Nitrogen as amides (precipitable by } mercuric acetate }	0.052	0.182
Nitrogen as amides (not precipitable } by mercuric acetate }	0.154	0.352
Total soluble nitrogen	0.355	0.642

Hilger and Van der Becke give the following figures of the soluble nitrogenous constituents of a barley and of the malt made from it, all of which are calculated on the dry substance :

—	Nitrogen of Albumin.	Nitrogen of Peptone.	Nitrogen of Ammonium Salts.	Nitrogen of Amido- Acids.	Nitrogen of Amides.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Barley	0.0600	0.0046	0.0169	0.0417	...
Barley steeped	0.0354	0.0009	...	0.0294	...
Green malt	0.1671	0.0058	0.0290	0.1417	0.0505
Dried malt	0.1194	0.0233	0.0057	0.2257	0.0029

HOPS.

Hops give to beer that pleasant, bitter, aromatic flavour which characterises it from all other alcoholic beverages. They have, however, several other important functions to fulfil. During the boiling process they precipitate albuminoids, and so assist in the clarification of the wort, and, by virtue of the resins which they contain, contribute essentially to the production of a stable beer. Hops are not only indispensable on account of these preservative properties, but also for the peculiarity of the bitter which they contain. This is of a transient nature ; that is, so soon as a beer flavoured with hops is swallowed, the sensation of bitterness immediately passes away, whilst with almost all other vegetable bitters the taste is persistent, and clings to the

mouth for a considerable length of time. The aromatic quality of the hop is essentially distinctive; although many other plants possess aromatic flavours, none of them exactly resembles that of the hop. Hops possess distinctly narcotic properties, and these appear to be greatest in the coarser varieties.

The Hop Plant.—The *Humulus lupulus* of the botanist is a perennial climbing plant, often met with in the wild state. It belongs to the *Cannabaceæ*, a small order of plants which includes the hemp, but is sometimes classed with the *Urticaceæ* or Nettle family. It is dioecious, that is, the male flowers appear on one plant and the female on another. In English and American hop-gardens it is usual to grow a sprinkling of male plants, but on the Continent these are for the most part excluded. In the former case the female flowers become impregnated and form seeds; in the latter they do not. It is thought by some that seed formation ought to be prevented, since the seeds are useless to the brewer, and only add weight to the hops. On this point experiments have been made by A. Howard, but no definite conclusion up to the present has been deduced from them. Hop plants are not raised from seeds, but by cutting off and transplanting portions of their underground stems. They are proverbially a risky crop, are subject to the attack of numerous insects and of various mould fungi, and are very susceptible to climatic conditions. Consequently, throughout the whole period of their growth they need great care and attention, such as constant washing to keep off insects, and sulphuring to keep off mould; and often, after the expenditure of an immense amount of trouble and care, a promising crop may be completely ruined by a few weeks of unpropitious weather just before the hop harvest.

The ripe female flowers are the parts of the hop plant used in brewing. Each of these consists of a number of bracts or small “petals” attached to the extremity of the stem in such a way as to form a cone. The bracts, which are shaped something like roofing tiles, are from a half to three-quarters of an inch in length, and each hop cone contains from twenty to forty of them. The lower portion of each bract is twisted on itself, and in the interior of this fold the female flower develops, to be followed later on by the seed.

The fruit of the hop is a little hard corn, about eight to twelve hundredths of an inch in diameter. Fig. 14 exhibits one of these fruits in the ripe condition; Fig. 15 the same with the exterior skin removed; and Fig. 16 the embryo taken out of the seed-vessel. The opinion that there is a relation between the size of the fruit and the quantity of flour which the hop contains is erroneous.

The peculiar yellow dust known as the hop-flour, or the “lupulin” which is contained in greater or less quantity in all hops, is of great importance to the brewer, for it chiefly contains those resins, volatile oils, and bitter substances which are of value. The lupulin is secreted by certain glands which are found on the inside of the bracts of the hop cone, upon the stem which bears the fruit, and upon the outside of the fruit. The quantity of lupulin varies considerably in different varieties of hop, and also in the same kind of hop

in different years, even when grown in the same district. The respective proportions of the different constituent parts of the hop cone, mechanically separated, are given by Haberland as follows:

Lupulin (hop-flour)	7.92 to 15.70
Bracts	69.79 to 78.36
Strig	8.50 to 17.54
Ripe seeds	0.02 to 7.80

In a sample from Wisconsin the last item amounted to 20 per cent.

In practice, the quantity of the hop-flour present—the “condition,” as it is called—is roughly estimated by rubbing the hops between the hands, the

FIG. 14.

FIG. 15.

FIG. 16.

Seed of the Hop.

amount of stickiness left forming an index as to the quantity of lupulin present in the hops.

Varieties of Hops.¹—J. Percival divides English hops into three classes—early, mid-season or main crop and late varieties respectively. The early sorts, as a rule, possess red bines, yield a comparatively small crop, and the quality is generally inferior. They include “*Prolifics*,” *Meopham*, *Bramblings*, &c. The mid-season or main crop include *Fuggles*, which is a useful copper hop of second-rate aroma, and the choicer varieties, such as *Canterbury*, *Golding*, *Mathon*, and *Farnham*. Among the comparatively few late varieties may be mentioned *Colegate*. Hops are cultivated most extensively in Kent, but also in Sussex, Surrey, Hampshire, Worcestershire, and Herefordshire.

The best known foreign hops include *Spalt*, and *Holletau* (Bavaria), *Saaz* (Bohemia), *Wurtemberg*, *Alsace* and *Lorraine*, *Alost* and *Poperinghe* (Belgium), *Burgundy*. The American hops from California, and more recently from British Columbia, deserve mention as useful sorts.

Physical Characteristics of Hops.—The relative value of hops may be determined by certain physical characteristics; J. Percival has dealt with these.² The lupulin glands contain the most important constituents of the hop; they are the structures sometimes spoken of as hop-meal, hop-flour, pollen or “condition,” and they originate as outgrowths on the small corolla surrounding the “seed” and on the bracts or “petals” of the hop cone. By the end of July or early in August the glands appear as small cup-shaped structures, which become more and more filled with oily resinous contents as the season advances. The oily material is for a time transparent and of a golden-yellow colour, but when the glands are quite ripe the contents become

¹ *The Hop and its Constituents*. Edited by A. C. Chapman, 1905, p. 6.

² *loc. cit.*, p. 10.

opaque and have much the appearance of "flowers" of sulphur. Hops are generally picked unripe, and even if allowed to ripen, the glands on being subjected to the drying process become again transparent; they have, therefore, invariably this appearance in commercial samples.

The characteristic aroma of hops, their preservative value, and flavour are almost entirely due to the contents of these glands. The aroma is chiefly due to the essential oil, whilst the preservative effect and bitter flavour are associated with various resinous substances dissolved in the oil.

There are two kinds of bracts or "petals" arranged on an axis or strig, which is branched. One kind of bract has the fruit or "seed" of the hop attached to it, whilst the other is seedless. The seed-bearing bracts have always a good number of glands present on their outer surfaces, but it is only in the better class of hops that lupulin glands are met with on the seedless "petals." On the corolla surrounding the "seed" they are very abundant. It is due to the recognised importance of these structures that some brewers have begun to show a decided preference for whole hops. Considerable amounts of lupulin are lost during the process of drying and bagging, and, moreover, rough treatment is liable to rupture the glands and set free the resinous contents, which are acted on deleteriously by the air. Broken hops do not retain their preservative power and aroma so long as whole ones. It is, therefore, the brewers' interest to lay more stress on whole hops than he does at present, and to modify his views on delicacy of colour, which leads growers to deliberately break up the hops after drying. The presence of many loose strigs and broken bracts are sufficient indication of a badly mutilated growth, and should be allowed for in estimating the value of samples.

Microscopical examination of the glands affords a means of distinguishing good hops from bad ones. In fresh hops the glands are of a pale golden-yellow colour with slightly wrinkled surfaces, and when pressed on a slide glass or rubbed on paper the oily contents ooze out; with age they become more wrinkled and darker in colour, assuming, finally, a deep reddish-orange; in samples dried at too high a temperature similar changes in shape and colour are noticeable. The resinous substances present in the glands lose much of their oily, sticky character when kept. Fresh hops, rich in lupulin, stick together, and slowly open out again when pressed tightly in the hand, although, as a whole, a properly dried sample is very elastic and springy when pressed.

The size and thickness of the strig, bracts, and seeds should be reduced to a minimum, and only the smallest leaves of the hop plant should be permissible in commercial samples. The bracts should be arranged at very close intervals along the strig and the latter should be fine and slender. Some sorts have 20-25 per cent. more bracts than others for the same length of "strig." Fully developed seeds should be few in number, for, apart from their increasing the weight of ineffective material, they are said to produce an objectionable flavour in the beer.

Hops of the best quality combine richness in lupulin, with excellence of aroma and flavour, and possess thin smooth bracts with slender veins; the colour is uniform all over such hops, both seed-bearing and seedless bracts being similar in tint. Inferior hops have coarse stiff bracts with prominent strong veins, which are not so much branched as in good varieties; the bracts are more wrinkled, long, narrow, and pointed at the tips, whilst in the case of the best hops the tips are rounded. The colour of low-grade hops is generally darker than is that of those of good quality, the petals at the base of the cone being frequently of a dirty green tint and somewhat twisted instead of flat.

The amount of lupulin on a hop "petal" does not increase proportionately with the size of the latter; the best hops are chiefly of the medium size, both large- and small-coned varieties being usually poor in lupulin. However, in the case of Continental hops some of the smaller-coned sorts are especially rich in lupulin.

Flavour and colour are both important in hops intended for the finest pale ales; inferior varieties possess a garlic odour, whilst samples which have been damaged in the drying process have a gingerbread or sweet odour. The odour is best determined on a sample rubbed down in the hand. Old hops stored in the usual way develop a cheese-like odour; cold-stored hops preserve their good qualities for a longer period, but must be used immediately after removal from the store, as they deteriorate rapidly.

The aroma of hops is of comparatively small moment except for dry hopping, but objectionable taints are often indicative of deterioration of other qualities of more serious import. When, however, preservative power is chiefly aimed at, hops of inferior aroma may be useful or more so than those of better aroma, for they are frequently very rich in lupulin, and whatever imperfect aroma they possess is boiled off in the copper.

Drying and Sulphuring.—The hops, after being plucked, are dried in a kiln resembling in some respects the drying kiln of the maltster. Much care is needed during the process, or they may be easily damaged. When the moisture has been completely removed, sulphur is placed on the fire, and this has the effect of brightening the colour of the hops; the sulphurous acid evolved also acts as an antiseptic, destroying to some extent the germs of mould fungi and other organisms attached to them. The question as to whether the sulphuring of hops has a prejudicial effect on the wort during its subsequent fermentation has often been a theme for discussion. The bulk of the evidence, however, goes to show that it is practically harmless. Probably this is not the case with regard to the particles of sulphur which are left on the hops after the sulphuring for mildew. When powdered sulphur is mixed with yeast, it exercises no prejudicial effect upon its fermentative action, but causes, during the fermentation, the evolution of a small amount of the offensive gas, hydrogen sulphide. Percival¹ states that unsulphured or unbleached samples would not sell readily, although the reverse holds on the

¹ *Loc. cit.*

Continent. The sulphuring may be of some real use in preventing the resins and oils from oxidising or in destroying colouring matters which affect pale ales deleteriously. The practice was originally adopted, however, to give the hops a fictitious colour. Thoroughly sulphured hops when picked very green have a colour attractive to buyers, and growers have frequently been advised to sulphur more extensively during drying. Percival is of opinion that in judging hops the question of colour should be kept in the background; in fact, exceptionally pale and delicate-looking samples he would suspect as unripe or as belonging to a silvery wilding race containing little lupulin. The colour of the small green leaves of the hops in commercial samples is useful as a guide to good or bad drying. In samples properly dried these leaves are of a fresh lively green colour, similar to a healthy leaf freshly picked; in coddled or burnt hops they are of a dirty brownish- or olive-green.

It has been shown by J. L. Baker and W. D. Dick¹ that when sulphur is thrown on to the fire the arsenic which might otherwise remain fixed in the fuel ashes is volatilised and contaminates the hops. This may be obviated by burning the sulphur out of direct contact with the fuel; but under these conditions there is a possibility of free sulphur being deposited on the hops. Special furnaces have, therefore, been devised in which the sulphur is completely burnt apart from the fuel and the sulphur dioxide produced passed through the hops.

The Chemical Constituents of Hops.—Hops contain, in addition to the ordinary constituents of plants, such as cellulose, woody fibre, &c., several other substances which are of the greatest importance to the brewer. These are the essential oil, the resins, the tannin, and the diastase.

Essential Oil.—On this constituent depends the characteristic agreeable aromatic flavour of the hop. The so-called lupulin or “hop-flour” contains resins, bitter principles (perhaps one or more alkaloids), wax and essential oil. The latter, although of higher boiling-point than water, is readily volatile with steam and can, therefore, be obtained from the hop strobiles (or from separated lupulin) by distilling these with water. It is found floating on the surface of the aqueous distillate. The strobiles yield 0.2 to 0.5 per cent. of this oil, which is a colourless or pale yellow mobile liquid, possessing a fragrant odour and a slightly burning taste. It is almost insoluble in water (to which, however, it imparts its characteristic odour), and is only slightly soluble in dilute alcohol. Hop-oil is readily soluble in alcohol and ether, but only slightly so in water, one part of hop-oil dissolving in from 600 to 700 parts of water. The boiling-point of the essential oil is about 125° C. (257° F.). Payen and Chevalier² prepared the oil by distilling lupulin with water. Wagner³ stated that it contained a terpene and an oxygenated constituent “valerol.” Personne⁴ submitted the oil to fractional distillation, but he did

¹ *Journ. Soc. Chem. Industry*, 1904, 23, 174. ² *Journ. Pharm.*, 1882, 8, 214 and 533.

³ *Journ. f. pr. Chem.*, 1853, 58, 351.

⁴ *Compt. rend. de l'Acad. des Sciences*, 1854, 38, 309.

not elicit much information thereby. Ossipow¹ studied its oxidation products by chromic acid and identified acetic and isovaleric acids among these.

The fact that most of the compounds occurring in essential oils were then unknown, retarded the advance of our knowledge of hop-oil, that great and important branch of organic chemistry which deals with the terpenes and their derivatives being of quite recent birth. The researches of Wallach and his colleagues induced A. C. Chapman some years ago to undertake a study of the essential oil of hops, with the help of the more refined methods which are now at our disposal. In the course of this investigation he worked with seven different samples of oil, which were prepared from hops grown in different parts of the world and in different years—two samples by himself and the remainder by Messrs. Schimmel & Co.

These samples were submitted to prolonged fractional distillation under reduced pressure, practically the same results being obtained in each case. The results are described in detail in various papers published by Chapman.²

Humulene, $C_{15}H_{24}$, a sesquiterpene, representing the largest fraction (about 40 per cent. of the oil), and the highest boiling portion of the oil, when pure has very little odour, and has therefore very little effect on those properties of the oil which are of chief technical importance; it may therefore be regarded as a diluent. The other constituents are myrcene, linalool, linalyl isononoate, with small quantities of a diterpene, and probably traces of some ester of geraniol; the hydrocarbons myrcene and humulene are present to the extent of 80 to 90 per cent.

It is well known that marked differences exist between the aroma of hops grown in different parts of the world, and that these are resident in the essential oils. No one, for example, relying on the sense of smell alone could mistake Californian for Bavarian hops or the latter for the produce of Kent. It is interesting in this connection to note that these variations, readily recognised as they are by the nose, yet correspond with such slight differences in chemical composition that the methods available for the separation of the constituents are altogether insufficient for the purpose of detecting them. The Californian oil, for example, yielded the same compounds as the Bavarian and English oils, and the difference in their odour must have been due partly perhaps to the different proportions in which the above constituents existed, but also, probably, to the presence of very small quantities of other fragrant substances, the separation of which in a state of purity was rendered impossible by the nearness of their boiling-points and by the great expense attending work with large quantities of the oil.

By the older authorities it was stated that the oil acted both as a flavouring and as a preservative agent, and that its rôle was consequently a most

¹ *Journ. pr. Chem.*, 1883 [2], **28**, 447.

² *Journ. Chem. Soc. Proc.*, 1893, **9**, 177; *ibid.*, *Trans.*, 1895, **67**, 54 and 780; 1903, **83**, 505; *Journ. Fed. Inst. Brewing*, 1898, **4**, 224.

important one, but A. C. Chapman and Burgess showed¹ that the oil has no antiseptic powers.²

Chapman's experiments showed that when the hops are boiled with water for half an hour, 80 per cent. or more of the oil has volatilised with the steam, the remainder being converted into a resinous mass. It can, therefore, be regarded as certain that little or no unchanged oil can remain in the copper wort after boiling. The hop flavour communicated to the wort during boiling is due chiefly to the resin formed by the oxidation and polymerisation of the oil, this resin (which must not be confounded with the preservative resins) being slightly soluble in the boiling wort and not volatile with the steam. It is therefore not improbable that there are also present in the hop cones solid, non-volatile substances which also communicate flavour to the wort. In dry hopping the oil plays a more obvious part, a little of it passing directly into solution in the beer. Resinification of the oil is retarded, but not prevented, by cold storage, it being much more dependent on the presence or absence of air.

Oxidation of Hop Oil.³—It will be seen that the essential oil of hops plays a less important part in the brewing process than was supposed by earlier investigators and authors. It must not, however, be thought that for this reason the character of the oil as indicated by the aroma of the hops is a point which the brewer may neglect, since the non-volatile flavouring resin, to which reference has been made, develops to a very considerable extent the peculiarities of colour exhibited by the fresh oil itself. There is very little doubt that any unpleasantness in the oil would be communicated to the resin, and so in turn to the beer. It is, moreover, one of the surest guides to the general value of a sample of hops, where reliance is placed solely upon a physical examination.

Bitter Principles and Resins.—The fact that lupulin contains a bitter substance was first pointed out by Ives,⁴ and this substance was prepared by Pellitan, Payen and Chevalier⁵ by treating an aqueous hop extract with lime and alcohol, evaporating the alcohol, extracting this residue with water, again evaporating, and extracting with ether; the aqueous solution of this yellowish-white substance has a neutral reaction. In 1847 Döbereiner⁶ stated that the substance was soluble in five parts of boiling water, more soluble in alcohol, and almost insoluble in ether; it was said also to be decomposed on heating with the formation of ammoniacal products. Personne⁷ claimed the discovery of a bitter substance of an alkaloidal nature, but failed to isolate it in a pure state. J. C. Leuchs⁸ endeavoured to remove the bitter substances from hop extract in various ways and succeeded on addition of acetic acid, tartaric acid, lemon-juice, sulphurous acid, aldehyde or formic acid, whilst the same result was attained by prolonged exposure of the extract to air, at

¹ *The Brewers' Journal*, 1896, **32**, 238.

² Compare also Briant and Meacham, *Journ. Fed. Inst. Brewing*, 1896, **2**, 420.

³ See *Journ. Fed. Inst. Brewing*, 1898, **4**, 224-233.

⁴ *American Journ. of Science*, 1820, **2**, 302.

⁵ *Journ. de Pharm.*, **8**, 209.

⁶ *Deutsches Apothekerbuch*, **3**, 636. ⁷ *Compt. rend. de l'Acad. des Sciences*, 1854, 309.

⁸ *Journ. pr. Chem.*, 1857, **101**, 137.

55–60° F. Vlaanderen¹ precipitated the resin from the alcoholic extract by the addition of water, the precipitate being taken up with alcohol and again precipitated with water. The resin obtained after repeating this operation several times was dark brown and tasteless, almost free from ash, soluble in alcohol and in ether, and had the composition $C_{54}H_{70}O_{11} + 1$ to 6 H_2O . Copper compounds $C_{54}H_{70}O_{11}$, CuO ; $C_{54}H_{70}O_{11}$, $2CuO$; and $C_{54}H_{76}O_{14}$, CuO were prepared.

Lermer² isolated hop bitter by extracting fresh hops with ether, treating the residue from the ethereal extract with 90 per cent. alcohol, whereby the wax remained undissolved, and taking up the residue from the alcoholic extract with ether. The ethereal solution was shaken with strong potash lye, whereby the bulk of the non-crystallisable resinous substances passed into the aqueous liquid. The ethereal extract was then repeatedly shaken up with water when the hop bitter passed into the latter, and was isolated by adding copper sulphate to this aqueous solution, a blue precipitate, consisting of microscopical needles, being formed, which was washed with small quantities of ether; large amounts dissolve it. Sulphuretted hydrogen was passed through the ethereal solution of this copper compound when the bitter substance itself passed into the ether, and on evaporating the solvent in a current of carbon dioxide a syrupy residue was obtained which solidified to a crystalline mass in 12–24 hours. By treating this mass with nitrobenzene white crystals were obtained, which became resinous and coloured on exposure to the air. Lermer states that it forms brilliant prisms, having no bitter taste on account of its insolubility in water; the solution in dilute alcohol is, however, distinctly bitter. The crystals are soluble in alcohol, ether, chloroform, carbon bisulphide, benzene and turpentine. The copper salt on analysis was found to have the composition corresponding with the formula $C_{32}H_{50}O_7$, CuO . Lermer refers to several other substances which he isolated from the hops, one of which forms silky needles melting at 110° C.

Etti³ found that ether extracts the oil, chlorophyll, wax, and an amorphous brown resin. When the residue from the ethereal solution is taken up with alcohol the resin is dissolved, and this may be precipitated on adding water to the alcoholic solution, leaving the bitter principle in solution; when this solution is evaporated over concentrated sulphuric acid colourless crystals are obtained, which are soluble in water and have a strong bitter flavour. Etti therefore regarded the bitter substance and the resin as distinct compounds.

Isslieb⁴ ascribed the formula $C_{29}H_{46}O_{10}$ to the hop bitter, and showed that on hydrolysis with sulphuric acid it yielded lupulieretin, $C_{10}H_{16}O_4$, and lupulinic acid, $C_{48}H_{82}O_{19}$.

Otto⁵ confirmed Lermer's results in general.

H. Bungener⁶ obtained a crystalline substance by extracting lupulin with petroleum ether. It was insoluble in water but soluble in most organic

¹ *Jahresber. f. Chem.*, 1858, 448.

³ *Zeits. ges. Brauw.*, 1878, 293.

⁵ *Zeits. ges. Brauw.*, 1880, 201.

² *Dingler's Polytech. Journ.*, 1863, 169, 54.

⁴ *Archiv. Pharm.*, 1880, 216, 345.

⁶ *Bull. Soc. Chim.*, 1886, 45, 487.

solvents, and its alcoholic solution had an intensely bitter taste. It melted at $92-93^{\circ}\text{C}$., and he ascribed to it the formula $\text{C}_{25}\text{H}_{35}\text{O}_4$ ¹. It had the properties of an aldehyde and of a weak acid, and on oxidation it yielded valeric acid. Gresshoff² failed to obtain a crystalline compound by Bungener's method.

Hayduck³ extracted hops with ether and isolated three readily distinguishable resins, two of which were bitter and the third tasteless; these he distinguished as α -, β -, and γ -resins respectively. From the α - and β -resins Hayduck obtained crystalline acids; the γ -resin he supposed was derived from the hop-oil.

Lintner and A. Bungener⁴ isolated Hayduck's acid, through the medium of the lead salt, in small rhombic crystals. H. Bungener's β -acid crystallises in long massive prisms or fine felted needles.

Seyffert⁵ obtained the α -hop bitter acid in a pure state by means of ice-cold petroleum. In collaboration with V. Antropoff he improved the method for the preparation of the β -acid, laying great stress on working in exclusion of air (in an atmosphere of carbon dioxide) and the avoidance of high temperatures. The various preparations had very ill-defined melting-points, which differed from that observed by H. Bungener ($92-93^{\circ}\text{C}$.), and analysis indicated that they were not homogeneous. The authors asserted that Lerner's acid was not identical with the α -acid, but belonged to a distinct class of substances, although it had faintly acid properties. They regarded hop resins as a mixture of substances undergoing continuous decomposition.

From what has preceded it will be seen that the chemical nature of the bitter substances of hops remained unknown, the elementary composition of the bitter acids and their molecular weights being by no means settled. Two crystalline acids had been separated from hops and three resins, two of which were bitter and one tasteless. As it was highly probable that the bitter resins were closely related to the crystalline bitter acids, these latter may be distinguished as the α - and β -acids. H. Bungener's acid is the β -compound, and the empirical formula assigned to it by him is probably correct. Lerner's acid was also probably the β -compound.

Lintner and Barth⁶ obtained the β -acid in large crystals by employing methyl alcohol as solvent; they found its composition to be correctly expressed by the formula $\text{C}_{25}\text{H}_{36}\text{O}_4$. This acid they refer to under the name of "lupulinic acid." On exposure to the air it oxidises, being converted into a yellow resin, and developing the odour of fatty acids. The acid maintains its properties when kept at a low temperature, which justifies the cold storage of hops in practice. When boiled with water it yields a bitter solution only if air is bubbled through the liquid, evidently because it polymerises to a resin soluble in water. The products obtained by Vlaanderen are doubtless identical with the β -resin. Whether the hard and soft resins of Briant and Meacham

¹ This formula should be doubled, as it contains an uneven number of hydrogen atoms.

² *Inaug. Diss.*, Jena, 1887.

³ *Wochensch. Brau.*, 1888, 937.

⁴ *Zeits. ges. Brauw.*, 1891, 357.

⁵ *Ibid.* 1892, Nr. 4, 5 and 6.

⁶ *Journ. Fed. Inst. Brewing*, 1900, 6, 514.

belong to this class of resins has not been decided. It is questionable whether they are chemical entities or mixtures of various resins with hop-oil. Lupulinic acid yields valeric acid on oxidation, but no definite products could be obtained on reduction. The copper salts prepared were not of constant composition. Sulphurous acid forms an unstable compound with lupulinic acid. A study of the action of bromine and iodine on lupulinic acid indicated the existence of double carbon linkages and of one methoxyl group. When boiled with potash, fatty acids are produced, and when fused with potash, products identical with those prepared from hop-oil by Greshoff¹ and by Wagner² are obtained; hence it is probable that lupulinic acid is closely related to hop-oil.

Bamberger and Landsiedl³ conclude that the formula of the α -acid is $C_{20}H_{26}O_5$ or $C_{20}H_{30}O_5$. Fusion with potash indicated a relation between the acid and hop-oil. It is an unsaturated compound closely resembling the β -acid, but it differs from the latter in its physical properties and also by the fact that it gives a crystalline compound by the action of potash solution and sulphuric acid.

The source of the bitter principles of hops is the lupulin. Holzner⁴ distinguishes three kinds of glands, simple glands—head glands, and glandular scales the latter being the hop-flour or lupulin granules. Under the microscope they appear to be multicellular, shield-shaped scales, with conical or cup-shaped indentations resembling two cones placed base to base. During development of the gland a secretion collects in the indented portions, and the upper cuticle breaks away and forms the tender yellowish hood forming the upper cone. By the increase of the secretion, the gland, as a rule, is burst open at the surface of contact of the two cones. The glandular scales easily break off the stalk by which they are attached to their bases, and so it comes about that in the preparation of hops for the market a part of the lupulin falls out. By lupulin, however, is also understood the secretory contents of the gland. The name has also been applied to an unknown alkaloid occurring in hops.⁵

The contents of the lupulin granules consist of a mixture of different bodies, the bitter substances predominating.

A sample of lupulin containing 18.27 per cent. of total ash yielded 63.93 per cent. of ethereal extract and 36.07 per cent. of matter insoluble in ether. The portion soluble in ether consisted of wax, 0.18 per cent.; α -resin, 11.55 per cent.; β -resin, 43.31 per cent. and 8.72 per cent. of fat and oil, &c. The portion insoluble in ether consisted of ash (soluble in hydrochloric acid), 15.81 per cent.; ash (insoluble in hydrochloric acid), 2.75 per cent.; albuminoids 4.78 per cent.; pentosans, 2.34 per cent.; nitrogen-free extract, crude fibre, tannin, &c., 10.89 per cent.

The extracts obtained by ether or by petroleum ether were also free from

¹ *Inaug. Diss.*, Jena, 1887.

² *Journ. f. pr. Chem.*, **58**, 351.

³ *Zeits. ges. Braw.*, 1902, 461.

⁴ *Zeits. ges. Braw.*, 1877, 267; 1892, 337; 1893, 303; 1899, 87.

⁵ Griessmayer, *Dingler's Poly. Journ.*, 1874, 67.

nitrogen. The proportion of silica in lupulin varies from 10.91 to 20.24 per cent. Analyses by other authorities show that the composition of lupulin is very variable.¹

As regards the determination of soft and hard resin, methods have been described by Briant and Meacham² and by Remy³ according to whom the hops of the 1896 harvest were characterised by notably low content of soft resin. Certain experiments made by Remy as to the influence of kiln-drying on hops show that a considerable portion of the soft resin present in the fresh hops is converted into hard resin during drying in the air.

In the course of investigations made by Lintner and Barth relating to bitter principles of hops, an attempt was made to estimate the β -bitter acid by titration of a hop extract.

The tannin of hops is said to effect a readier coagulation of the albuminoids of wort, whilst hop resins give beer a pleasant flavour and enhance its keeping properties. The antiseptic power of hop resins was first proved by Hayduck⁴; Richardson,⁵ Morris,⁶ and Heron⁷ have contributed to it. Schönfeld⁸ found that an alcoholic solution of the soft resins of hops acts as a powerful poison towards *Sarcina*. Barth⁹ showed that the α -resin was less effective than the β -resin and that the various species of *Sarcinae* are differently influenced by the hop resins.

Lintner and Schnell¹⁰ have recently studied the α -hop bitter acid. They prepared this acid, approximately pure, in the form of a brown, crystalline mass by liberating it from its lead compound. The acid was subjected to hydrolysis by boiling with alcoholic soda; it thus yielded, in addition to resinous matters and valeric acid, a well-defined decomposition product to the extent of about 30 per cent., which crystallised in the form of colourless, elongated, tabular crystals, melting at 92.5° C. This product shows no signs of resinification, and is sparingly soluble in water, but readily soluble in most organic solvents. Analysis and determinations of its molecular weight indicate the formula $C_{15}H_{24}O_4$, and its reactions point to the presence of a carboxylic group, a ketonic or carbonyl group, and an ethylenic double bond; it is regarded as a hydroxyketonic acid. Since the α -hop bitter acid yields only valeric acid on oxidation by permanganate, it may be regarded as being composed of the above hydroxyketonic acid condensed with a valeric acid residue; its formula should therefore be written $C_{20}H_{32}O_5$. The authors propose the following nomenclature for the bitter principles of hops; lupulinic acid for the β -hop bitter acid, $C_{25}H_{36}O_4$; humulone for the α -hop bitter acid, $C_{20}H_{32}O_5$, since it is probably a lactone; humulinic acid or humulin for the above-mentioned

¹ Ives (*loc. cit.*), Payen, Pelletan, and Chevalier (*loc. cit.*); Aubry (Lintner's *Lehrbuch. d. Bierbrau.*, 1878, 96-98).

² *Journ. Fed. Inst. Brewing*, 1897, 2, 233.

³ *Wochensch. Brau.*, 1898, 55, 930; see *Journ. Fed. Inst. Brewing*, 1889, 5, 41.

⁴ *Wochensch. Brau.*, 1885, 268; 1888, 945.

⁵ *Journ. Inst. Fed. Brewing*, 1898, 4, 128.

⁶ *Trans. Lab. Club*, 2, 26; 3, 24.

⁷ *Diary of the Brewing Room*, 1889.

⁸ *Wochensch. Brau.*, 1898, 292; 1899, 665.

⁹ *Zeit. ges. Brauw.*, 1901, 233.

¹⁰ *Zeit. ges. Brauw.*, 1904, 27, 666-669.

hydroxyketonic acid, $C_{15}H_{24}O_4$, derived from humulone by hydrolysis with alcoholic soda.

Hop-Tannin Bodies.—Hop-tannin, which was first isolated in a fairly pure state by Etti, is contained in quantities of from 2.5 to 6.0 per cent. in hops. Etti extracted hops first with ether and then with absolute alcohol, by which solvents the hop-oil, the resins, and the fatty substances were removed. The hops were treated with dilute alcohol to dissolve the tannin, which was precipitated therefrom by an alcoholic solution of lead acetate. The precipitate thus obtained was decomposed by hydrogen sulphide, when the tannin was left in solution, from which, by subsequent filtration and evaporation, it was obtained in the solid state. Prepared in this way hop-tannin is a fawn-coloured powder, of the composition $C_{25}H_{24}O_{13}$, soluble in water and dilute alcohol. Its aqueous solution precipitates albumin, but not gelatin (see, however, p. 206). Etti also found that the hop-tannin was accompanied by another substance, phlobaphen, having the composition $C_{50}H_{46}O_{25}$. Hayduck¹ has recently examined both these bodies with great care. Working by Etti's method, he extracted hops with ether and absolute alcohol, and then with dilute alcohol. This latter solution, which contained the tannin and phlobaphen, was fractionally precipitated with alcoholic solution of lead acetate. The first fractional precipitate consisted chiefly of phlobaphen, the last of tannin, the others of mixtures of the two. The separation of the two bodies was affected by means of acetic ether, which dissolves the tannin and leaves the phlobaphen behind. The purified hop-tannin was obtained as a pale brown amorphous powder, soluble in water, insoluble in ether, and nearly so in absolute alcohol. Dissolved in water, it had a slightly bitter, afterwards somewhat astringent taste. It showed an acid reaction with litmus-paper, and gave a dark green coloration with ferric chloride, but no precipitate. It is absorbed by powdered hide, and this has an important bearing on its quantitative estimation. Especially remarkable is the readiness with which it becomes transformed into phlobaphen; even in the evaporation of a solution of hop-tannin part of the tannin suffers this change. If hop-tannin is heated in the dry state to $140^{\circ} C.$, or if its solution is evaporated with a little sodium carbonate, the whole of the tannin is quickly and completely converted into phlobaphen.

Phlobaphen was obtained in the form of a reddish-brown powder, only partially soluble in boiling water or in absolute alcohol. Its aqueous solution is of a brownish-yellow colour, and has a disagreeably harsh, astringent taste. It gives with ferric chloride a dirty dark-green precipitate. Albumin is precipitated by phlobaphen, and the latter is removed from its aqueous solutions by powdered hide. The most noticeable point with regard to this substance is that it forms with the albuminous matters of the wort a compound which is completely insoluble, whilst the tannin forms a compound which is soluble in boiling water or wort, and also soluble to some extent in these liquids when cold.

¹ *Wochensch. Brau.*, 1894, p. 409.

Alkaloids of Hops.—Lermer¹ isolated a basic nitrogenous substance from hops. Griessmayer detected the presence of an exceedingly small quantity of an alkaloid in hops, which he terms “lupuline,” and which is said to be a narcotic. It seems to be present only in the coarser varieties of hops, some of the finest specimens being quite free from it. He also obtained considerable quantities of trimethylamine by distilling an aqueous extract of hops with potash or magnesia. American hops are said by Williamson to contain 0.15 per cent. of an alkaloid which he has named “hopeine.” This, according to Williamson and Leuken, can be separated into two bodies by means of amylic alcohol, the one is identical in composition with morphine, and called by them “isomorphine,” the other being pure hopeine. Ladenburg² proved that the so-called “isomorphine” is actually morphine. Beer, when concentrated in a vacuum and partaken of, is said by Langer to exhibit a pronounced narcotic action similar to that caused by morphine. Another basic substance is the choline found in hops to the extent of 0.02 per cent. by Griess and Harrow. Griessmayer is of opinion that choline is not contained in the hops as such, but as a constituent of lecithin, a complicated fatty body also found in the brains of animals. Recently, Kolomon Farkas³ has shown that hopeine, when injected into the veins, acts as a strong cardiac poison; but that when administered by the mouth it is comparatively harmless.

Other Constituents.—Besides the constituents mentioned above, hops contain a gum, which is soluble in water; a soluble red colouring matter, which has an influence on the colour of the beer; proteïd bodies, and some other substances which are extracted on prolonged boiling with water, and which have a rank, coarse, disagreeable, bitter flavour. Hops, like all other plants, contain a certain amount of mineral matter, which chiefly consists of the phosphates of potassium and magnesium.

Chemical Analysis of Hops.—Although it is possible to estimate with a fair degree of precision the various constituents of hops, it has not been so far found possible to establish any very definite relation between the value of the hops and the amounts of essential oil, resins, and tannin which they contain. Consequently, up to the present time, chemistry has not afforded much assistance in this direction. Briant and Meacham⁴ have proposed a method for the valuation of the preservative power of hops, which consists in estimating the amount of the soft resins they contain. So far as can be seen at present, the method appears to be capable of yielding satisfactory results. Heron⁵ proposes to estimate the value of hops by the amount of tannin they contain; he considers that the larger this amount is, the better the sample. Barth⁶ confirms this view to a certain extent; he finds that all good hops contain high percentages of tannin, although there are cases in which hops of very different quality contain nearly the same quantities of tannin. Contrary to the statement of Etli (p. 205), both these observers

¹ *Chem. Centr.*—*Bl.*, 1863, 781.

³ *Archiv. Physiol.*, 1902, 92, 61.

⁵ *Ibid.* 1896, p. 164.

² *Ber. d. Deutsch. chem. Ges.*, 1886, 19, 783.

⁴ *Journ. Fed. Inst. Brewing*, 1897, 2, 233.

⁶ *Zeit. f. d. gesamt. Brauwesen*, 1897, p. 168.

find that hop-tannin is precipitated by gelatin, and that this substance is a much more efficient reagent in the estimation of the tannin in hops than hide-powder.

Diastase of Hops.—Hops, like all other plants, contain diastase, but this point appears to have been overlooked until attention was called to the fact by Brown and Morris.¹ The enzyme can only be extracted by water after removal of the tannin contained in the hops. A similar phenomenon was observed by Baranetzky² with reference to acorns. The presence of diastase in hops is important as regards its influence on the secondary fermentation in cask, since it there helps to degrade the higher maltodextrins and bring them into a state amenable to the action of the yeast.

Cold Storage of Hops.—Briant and Meacham³ described numerous experiments which they had conducted to discover the best method of storing hops. In some cases the hops were placed in air-tight iron cylinders, and the air exhausted; in others the air in the cylinder was replaced by nitrogen, or carbon dioxide, or sulphurous acid, but none of these processes was found to be more effective than the ordinary methods of storage. They became convinced that the deterioration was caused by micro-organisms and enzymes, and this induced them to try the effect of storing at a low temperature, which method proved entirely successful. After experimenting for a number of years, they found that desiccation of the hops was also an important factor in their preservation, so much so, that hops deprived of a portion of their normal moisture could be kept without deterioration, for any reasonable time, at a temperature of 40° to 45° F., provided that the surrounding air was kept dry. They recommend the use of a chamber, the air of which is dehydrated by means of calcium chloride (which can be re-dried by heat when necessary), and which is maintained at a temperature of 36° to 40° F. by means of refrigerating pipes. Cold storage of hops was suggested by Isslieb in 1892.⁴

Vacuum Storage of Hops.—A patent for a process of vacuum storage has been granted to M. Trier and A. Wilkinson (Eng. Pat. of 1900, 11,352). The apparatus is somewhat more costly than that required for cold storage; but the cost of up-keep is probably less, and apparently hops may be stored in the manner suggested for an indefinite period without undergoing any change; thus hops placed in the vacuum store in 1899, when taken out in 1903, were said to be identical in all respects with new hops.⁵

¹ *Trans. Inst. Brewing*, 1893, p. 94.

² Sachsse, *Agriculturchemie*, p. 364.

³ *Journ. Fed. Inst. Brewing*, 1897, 3, 487.

⁴ See Prior, *Chemie und Physiologie des Malzes und des Bieres*, pp. 268 and 272.

⁵ See *The Brewers' Journal*, 1901, pp. 6-8.

PART II

CHAPTER III

THE MICROSCOPE

THE microscope in its simplest form is merely a magnifying-glass, and, as such, has undoubtedly been known for a very long period. We meet with frequent references in ancient literature to burning-glasses, which are convex lenses; and as all such lenses possess the power of magnifying objects, this property could not fail to attract attention and receive practical application. The compound microscope, which seems to have been invented in the sixteenth century, was at its birth, and for a long time afterwards, a very imperfect instrument. For a considerable period it seems to have been used for little else than for purposes of amusement and curiosity, such as the observation of small insects, until it was first practically applied by Leeuwenhoek in 1675 to purposes of scientific import. This pioneer in microscopical science found that whilst fresh water contained no living organisms, an infusion of ground pepper, which had been allowed to stand for a few days, teemed with them. He also expressed the opinion that these organisms did not arise spontaneously, but were developed from germs which previously existed in the atmosphere, and which had fallen into the liquid; he considered that the reason they grew abundantly in an infusion of pepper or other vegetable substance, and did not appear in pure water, was because there was an abundance of food in the infusion. Since then the question as to the origin of the living organisms which appear in this way in infusions has been the subject of many discussions and contentions, and these have ended in absolutely confirming the truth of Leeuwenhoek's remarkable surmise. This observer also recognised the peculiar forms of starch and pollen grains; he also observed that yeast consisted of a number of round or oval bodies. His observations were soon followed by those of Hook, Malpighi, and Grew, by whom the cellular nature of the tissues of living organisms was recognised. The microscopes used at that time were necessarily very imperfect instruments; the art of grinding lenses correctly to shape was not then understood, nor was the method of constructing a lens with two different kinds of glass, each having a different refractive power, and in this way getting rid of chromatic aberration. The images of the objects under observation were not only

indistinct, but all lines or points in them were bordered by a series of coloured rings. It was only at the commencement of the present century that these matters came to be better understood, the result being that in 1830 microscopes capable of magnifying up to 400 times, and of a much more perfect construction, were obtainable. From this time forward a list of the names of the microscopical workers who have enriched our knowledge, not only of the minute structure of animals and plants, but also of the processes going on in them, would in itself form a long catalogue. As time went on, the study of the lower organisms, such as the mould and yeast fungi, became themes for frequent investigation. Bail studied the organisms met with in fermenting worts, and surmised that there existed a family relationship between them and the mould fungi. The yeast fungi were also minutely investigated by Rees, and the mould fungi by Brefeld and De Bary. Pasteur extensively employed the microscope in his researches on fermentation. With his important discovery that many of the disasters of the brewery were caused by organisms foreign to the true ferment, yeast, and that their presence could be readily detected by the aid of the microscope, this instrument at once took its place amongst the other instruments of precision employed in the brewery, such as the thermometer and the hydrometer. The more recent investigations of Hansen have rendered the microscope of still greater importance to the brewer, for without its aid the cultivation and practical employment of single-cell yeast would be an impossibility. In more recent years the study of the bacteria, which are the smallest organisms met with in nature, has occupied much attention, and the results already obtained in this field are of immense importance. Continued investigation in it is likely to lead to discoveries the significance of which can be scarcely conjectured. Apace with this, and partly impelled by the necessity for increased perfection in the instruments specially designed for the observation of these minute organisms, such enormous improvements have been made both in the optical and mechanical parts of the microscope, that it is now possible to obtain colourless and absolutely distinct images of objects under magnifications of two or three thousand diameters.

Description of Microscopes.—Microscopes are of two kinds, the simple and the compound. The former in its most rudimentary form consists of a single lens; it is, in fact, simply a magnifying-glass. As such, it is occasionally used in the brewery for the examination of malt, hops, &c.

The compound microscope, which is, however, much more extensively employed by the brewer, consists of two portions, the optical and the mechanical. Of the two, the former is by far the most important, for though it is possible to do fairly good work with inferior mechanical arrangements providing the lenses are good, it is impossible to work with any degree of satisfaction if the optical portion of the microscope is bad. For comfortable, convenient, and accurate working, it is necessary that both portions of the instrument should not only be of good workmanship, but also specially adapted to the class of work for which it is

required. For this reason it is extremely desirable, when purchasing an instrument, only to go to first-class makers who guarantee their work, and also, if possible, to obtain the assistance of some one who is an adept at the instrument.

The mechanical portion of a microscope consists of a tube, A (Fig. 17), called the body-tube, upon the lower end of which, technically known as the "nose," an internal thread is chased, into which the various objectives are screwed in turn, or into a nose-piece which carries two or more objectives; this latter is shown attached at B. Into this body-tube there slides another tube, C, of slightly less diameter, termed the "draw-tube." This is completely open at its upper end, and into this opening the various eye-pieces are inserted. By means of the draw-tube, the length of the instrument can be varied; this alters the distance between the eye-piece and the objective. The body-tube is moved up and down by rotating the large milled heads, D, and by this movement the "coarse adjustment" of the distance between the objective and object is effected. The pillar, E, has a triangular bore, and slides on a triangular pillar, the movement being effected by an exceedingly fine screw actuated by the button, H; this arrangement is called the "fine adjustment." The triangular pillar is fixed to the stage, I, which is usually a perfectly level brass plate, perforated with an aperture immediately under the objective; it serves to support the object. The stage is attached by a movable joint, K, to the pillar, L, which, in its turn, is fixed to the horseshoe-shaped foot, M; this latter is generally loaded with lead in order to render the instrument stable. Under the stage the tail-piece, N, is fixed, which on its lower extremity carries the half-circle in which the mirrors, P, swing. The semicircular piece is attached to the tail-piece by a screw in such a way that it can revolve on the axis of the screw, and this motion, combined with the swinging one, enables the mirrors to be placed at any required angle. The substage, O, which can be raised or lowered by the rack and pinion, R, is intended to support the condenser and other accessory apparatus. A series of diaphragms, perforated by apertures of different size, and which can be fixed under the stage, are also provided. These are not required when an "Iris" diaphragm is provided (see p. 213).

The microscope is often constructed so that the coarse adjustment is effected by the body of the instrument sliding in a tube, and this arrangement is sufficient for the ordinary work which the brewer has, as a rule, to carry out; yet, when really fine work has to be done, the rack-work adjustment is almost a necessity, since by its employment a more accurate centering of the instrument is retained.

The length of the body-tube varies considerably in the microscopes of different makers; the English ones provide a wide tube, 10 inches in length; the Continental makers a narrower tube, 6 inches in length. This difference in length becomes a matter of considerable importance if the observer uses both English and Continental objectives, since the former are optically corrected for the long tube, the latter for the short one. In such a case it is

better to have a microscope with a 6-inch tube, which can be extended to 10 inches by means of the draw-tube. The shorter and smaller instruments are

FIG. 17.—Microscope (Leitz's Stand Ia, with triple nose-piece and Abbé's Condenser).

undoubtedly more convenient to handle, and can be worked with a greater degree of comfort than the larger instruments, which are somewhat unwieldy. In the instruments with the shorter tube, the hands, when moving the object

about on the stage, are nearer the eye; and delicate movements, such as those required for moving the object about on the stage, are more easily performed under these circumstances than when the hands are a considerable distance away from the eye. Since this fact has become universally recognised, most of the English makers manufacture instruments with the body-tube of the Continental length.

The nose of the microscope should be provided with a screw-thread of the pitch known as the "Microscopical Society's standard," as all English and most Continental objectives are provided with this standard thread. In case an objective is not fitted for this gauge, an adapter which will enable the objective to be screwed on to the nose of the instrument can be procured from the optician at a trifling cost.

Nose-Piece.—This arrangement (Fig. 17, *B*) screws on to the nose of the microscope, and is made to carry two, three, or more objectives. These can be successively brought into line with the instrument by revolving the lower plate of the nose-piece, which is then secured in position by a catch. This appliance is a great convenience, and saves much time which would otherwise be lost in screwing and unscrewing the objectives on and off. The objectives, when attached to the nose-piece, are covered and protected from dust.

The Stage.—This, in its simplest form, consists of a perfectly level brass or glass plate mounted so as to be at right angles to the axis of the body of the microscope. In its centre is a circular opening for admitting light to the object. A pair of spring clips serves to keep the glass slip closely applied to the stage, whilst, at the same time, these allow it to be freely moved about; for most purposes these are all that is necessary. Some stages are provided with a sliding bar, which can be moved to and from the observer, the bar always maintaining a position at right angles to the sides of the stage. Some microscopes are provided with a mechanical stage, by means of which the object can be moved in two directions at right angles to each other. This arrangement adds considerably to the cost of the instrument, and for the purpose of the brewer is quite unnecessary. With a plain stage the hands soon become accustomed to move the object about with the greatest degree of nicety. All objects when seen under the microscope appear upside down, and consequently all movements of the object appear reversed. This reversal of the movements of the object causes a little difficulty at first, but, with practice, this is soon overcome.

The Diaphragms.—These consist of thin plates of metal perforated with apertures of various sizes, and are attached to the stage in various ways; they serve to regulate the amount of light which obtains access to the object. The diaphragms are often attached to short cylinders which accurately fit into the circular opening in the stage; or the cylinders may be attached in turn to a plate which slides into a dovetailed groove underneath the stage. These forms are often replaced by a circular plate of metal, perforated near its margin by circular apertures of varying sizes, which is fixed under the

stage by a screw passing through its centre, around which the plate rotates. The apertures, as the plate is revolved, successively come into position, each being then exactly in the axis of the microscope; a little catch retains the plate in this position. This is not such a good arrangement as the former, for to obtain the best effects the diaphragm should be immediately under the object. In some instruments the diaphragm-plate is attached to the sub-stage, and is then a considerable distance below the stage; this is the worst form of all.

Iris Diaphragm.—An exceedingly convenient form of diaphragm called the “iris” diaphragm is obtainable. It is provided with a number of plates of a peculiar form, so slotted and pivoted that when these are actuated by a lever the aperture in the diaphragm is widened or contracted.

Sub-Stage.—This is an arrangement underneath the stage, which generally consists of a brass ring of about $1\frac{1}{2}$ inch in diameter, fitted to the tail-piece of the microscope in such a way that it can be moved to and from the stage. This movement is often obtained by a slide working in a dovetailed groove, but it is much better effected by a rack-and-pinion movement. The principal use of the sub-stage, so far as the work of the brewer is concerned, is to support the condenser. The best instruments are provided with an arrangement for bringing the axis of the condenser accurately into line with that of the body of the instrument. In the instruments of Zeiss the condenser is hung on a side-pin attached to a piece which slides up and down the tail-piece; this permits of the condenser being temporarily turned out of the way when not wanted, and it can be completely removed by simply lifting it off the pin.

Cheaper instruments have, instead of a sub-stage, a short plain tube fixed under the stage, into which the condenser slides; the tube should be fitted so as to be truly central, or it is comparatively useless.

Objectives.—These are by far the most important portion of the microscope, for on their perfection depends, in the greatest measure, the clearness and distinctness of the image viewed. Great care should, therefore, be exercised in their choice. Objectives generally consist of three small achromatic lenses mounted in brasswork, the upper portion of which carries an external screw-thread by which the objective is attached to the microscope. Each achromatic lens consists of two lenses of different kinds of glass cemented together; by this arrangement an image free from colour is obtained. Many lenses have what is called a “correction mount,” by means of which the front lens can be removed nearer or farther from the other two lenses by turning a collar. This arrangement is for making a correction for the thickness of the cover-glass employed. All objectives without a correction mount, homogeneous oil immersions excepted, yield their best results with a cover-glass of a definite thickness, and this is generally indicated on the objective. For instance, if an objective is marked 0.18, this means that a cover-glass of the thickness of 0.18 millimetre is to be used with that particular objective in order to obtain the best results with it. When, however, the objective is provided with a correction mount, the

observer is to a great extent independent of the thickness of the cover-glass; he has simply to focus the object, and turn the correction collar backwards and forwards until he finds the position which yields the best and clearest image. The correction mount is generally provided with an index, every division of which corresponds to a 0.1 of a millimetre thickness of cover-glass; consequently, if the thickness of the cover-glass is known, the index is simply set to the figure corresponding to that thickness.

A certain amount of correction for thickness of cover-glass can also be made with the draw-tube of the microscope: when the cover-glass is too thin, the draw-tube is to be pulled out; when too thick, it is to be pushed in.

The magnifying power of objectives is not, as a rule, directly stated, but their focal length is given instead. The magnifying power of the combination is equivalent to the enlarging power of a simple lens of the same focus. A simple lens of 1-inch focus has, for ordinary eyesight, a magnifying power of ten times, a half-inch of twenty diameters, and so on, the magnifying power being in inverse proportion to the focal length of the objective. An objective having a focal length of a quarter of an inch will, therefore, have a magnifying power of 40.

Aperture.—The amount of detail which an objective is capable of showing depends upon what is called the “angle of aperture” of the objective. Of two objectives of the same focal length but different apertures, that with the wider angle will permit the entrance of a larger cone of rays than that with the narrower angle; the former will consequently have a higher resolving power. Such objectives are considerably more difficult to manufacture than those of a narrow angle, and are consequently much more expensive; they are not, however, required for the purposes to which the brewer ordinarily applies his microscope. Moreover, they possess but little “depth of focus”; that is, the thickness of the plane which is in focus at one time is exceedingly thin, and the wider the aperture with objectives of the same focus, the thinner this plane becomes. The same happens with objectives of high power; the shorter the focus, the thinner the plane in focus. This is a source of some difficulty to the beginner, and is remedied by perpetually altering the focus of the instrument. In this manner a number of different planes are brought successively into view, and, by combining these mentally, a general conception of the structure of the whole thickness of the object is obtained. This cannot, however, be pushed too far, because, if the object under examination is thick, its deeper layers appear misty and indistinct.

Apochromatics.—A new series of objectives has been introduced of late years which are called “apochromatic.” By the employment in their manufacture of special kinds of glass, and the introduction of a lens of the mineral fluorite, and also by means of the most careful mathematical calculations, these objectives have been brought to a higher degree of perfection than any hitherto produced. The apochromatics are exceedingly expensive, and will

never be required by the brewer unless he intends to become an expert in bacteriology; even in this case he will manage for a long time with the ordinary objectives, since at first he would probably be unable to appreciate the difference between the two. The apochromatics possess one marked advantage; they can be used with eye-pieces of very high magnifying power. Thus a quarter-inch objective may be used with an eye-piece magnifying 27 diameters, which gives an enlargement of 1080 diameters, as well as with an eye-piece magnifying only 2 diameters, or with any other eye-piece intermediate between these two. In this way magnifications of from 80 to 1080 may be obtained by simply changing the eye-pieces, and much trouble is saved in screwing and unscrewing the various objectives.

Immersion Objectives.—In addition to the ordinary objectives, which are termed for the sake of distinction “dry,” another series is manufactured which are termed “immersion.” When using these, a drop of water is placed between the cover-glass and the front lens of a “water immersion” objective, or a drop of cedar-wood oil in the case of an “oil immersion.” The object of using an immersion liquid is to bring a larger cone of rays into the objective. Light in its passage from the cover-glass to a dry objective has to traverse a layer of air, and rays of light in passing from glass to air suffer refraction, that is, they are bent away from the perpendicular. If, however, a layer of a medium such as water, which has a higher refractive power than air, is inserted between the cover-glass and the front lens, then the rays are not bent so far away from the perpendicular, and consequently a larger bundle enters the objective. When a layer of cedar-wood oil, which has the same refractive power as glass, is inserted in this way, the rays suffer no refraction at all; they continue straight along in their course, consequently a still larger bundle is able to enter the objective. This is not only equivalent to increasing the angle of the objective, and thus giving better resolving power, but, as there is always a certain loss of light when the rays pass through layers of media of differing refractive powers, a gain in the direction of illumination is also obtained. It follows, as a consequence of the oil employed being of the same refractive power as the cover-glass, that an oil immersion is independent, within wide limits of the thickness of the cover-glass, and for this reason such objectives are called “homogeneous.”

Objectives Necessary for the Brewer.—For most purposes two will suffice, an inch and an eighth of an inch. The former will be found useful where low magnification is required, or for finding the position of small objects, and the second is sufficiently powerful for the examination of yeast, whilst bacteria are readily seen with it.

Should it be intended to enter more deeply into the study of bacterial organisms, a higher power will be required, and the most useful all-round objective for such work is a twelfth of an inch oil immersion.

Care of Objectives.—Microscopical objectives are very delicate and fragile articles; consequently great care must be taken not to subject them to any rough usage; a fall on to the floor would be almost certain either

to fracture the lenses or dislocate them from their settings. If the surfaces of any of the lenses, with the exception of the front surface of the lowermost one, get contaminated with dirt, the objective should be sent to the optician to be cleaned. After working with an immersion objective, it should be carefully freed from adherent liquid and polished with a soft cloth. A little chloroform or benzene will assist in the removal of the last traces of the cedar-wood oil used with oil immersions. When using an immersion objective, care must be taken that the immersion liquid does not get mixed with the liquid oozing from between the glass-slip and cover-glass; also, when observing objects mounted in Canada balsam, that the oil does not get contaminated with the balsam.

Eye-Pieces.—The most common and generally useful form of eye-piece is that invented by Huyghens, and called after him “Huyghenian.” It consists of two plano-convex lenses, having their convex sides directed towards the objective, with a diaphragm between the lenses. It is the simplest and least expensive form of eye-piece, and is suitable for all ordinary purposes; in fact, it is the best form to use with ordinary objectives, for, as these are over-corrected and the eye-pieces under-corrected, the two balance one another and give a correct image. When, however, apochromatic objectives are made use of, what is called “compensating” eye-pieces must be employed. These are over-corrected, and, as the apochromatics are under-corrected, the two balance one another. As compensating eye-pieces are constructed with achromatic lenses, they are considerably more expensive than the ordinary Huyghenian.

The various magnifying powers of the eye-pieces are denoted in England by the letters A, B, C, &c., beginning with the lowest power; on the Continent by numbers, 1, 2, 3, &c. The magnifying powers corresponding to these will generally be found in the maker’s catalogue.

With a microscope having a 10-inch tube, the total magnifying power of any combination is found by multiplying the magnifying power of the objective by that of the eye-piece. Thus, taking an eighth of an inch objective, which magnifies 80 diameters, and an eye-piece magnifying 5 diameters, we obtain a total magnification in diameters of $80 \times 5 = 400$. With a 6-inch tube the magnifying power is found by the formula:

$$\frac{\text{objective power} \times \text{ocular power} \times \text{tube length}}{10};$$

with the before-mentioned combination this would be 240 diameters $\left(\frac{80 \times 5 \times 6}{10}\right)$.

Illuminating Apparatus.—In work where objectives of no higher power than a quarter of an inch are employed, the light reflected by one of the mirrors under the stage will be sufficient to fully illuminate the object. One of these mirrors is plane (that is, perfectly flat), the other concave; the latter brings a conical bundle of light rays to a focus on the object, and consequently gives a greater illumination. The former mirror is employed when viewing

objects with low magnifying powers, the latter when higher-power objectives are being used. When, however, it is required to work with powers higher than the quarter of an inch, or with an immersion objective, it is absolutely necessary to employ a condenser which brings a very large bundle of light rays into the objective. The most common form of condenser is that invented by Abbé (Fig. 17, *O*), which consists of two or three convex lenses of differing curves. Since it is made with simple lenses, it is not achromatic; but as it is comparatively inexpensive and fulfils most ordinary requirements, it is frequently employed. Achromatic condensers are also made on a similar principle, and, though considerably more expensive, undoubtedly possess advantages over the cruder form. The condenser, whether of the achromatic or non-achromatic form, is either provided with a series of diaphragms, with a revolving plate, or with an iris diaphragm. An arrangement is provided on the sub-stage by which the condenser can be moved nearer or farther away from the object, the best form for this being a rack-and-pinion movement. An arrangement for centering the condenser truly is also necessary where fine work has to be performed.

One of the lower-power objectives, half- or quarter-inch, makes a very efficient condenser, if an arrangement is provided for attaching it to the sub-stage.

Choice of a Microscope.—For the ordinary work of the brewery, which chiefly consists in examining yeast, one of the smaller and less expensive forms of microscope stand is sufficient. These are to be obtained at prices ranging from £1 10s. to £5. Such a stand, fitted with an inch and an eighth of an inch objectives,¹ and a couple of eye-pieces magnifying five and ten times, will be ample for this purpose.

The microscope stand should be thoroughly rigid, stable, and free from all vibration; the form of foot such that when the body-tube of the instrument is placed in a horizontal position there is no tendency to topple over. All the movable parts should work smoothly and without any tendency to jerkiness, and should fit so accurately that the body of the instrument, wherever placed, is never thrown out of line. This should be especially the case with the fine adjustment. When an object is being focussed, either with the coarse or the fine adjustment, it should not change its position in the field.

Objectives.—These should give a bright, well-illuminated field; the image of the object should be clear and distinct, without the slightest tendency to haziness; its contours should be cleanly cut, and not surrounded by fringes of various colours. In testing objectives it is usual to employ what is called a "test-object." This, for objectives up to $\frac{1}{4}$ th of an inch, generally consists of the scales of the wings of moths, many of which exhibit delicate markings. The *Pleurosigma angulatum*, a diatom which is readily obtainable, forms an excellent object for testing the higher objectives. Its

¹ A $\frac{1}{16}$ -inch objective in addition to those above mentioned will also be found useful.

markings should be distinctly brought out by the $\frac{1}{8}$ -inch objective, their honeycomb formation being rendered distinctly visible.

The field of view should be perfectly flat. When a perfectly flat object, such as the impression of the tip of the finger on a glass slip, is viewed, the whole impression should be in focus at the same time. This should, at all events, be the case with the lower objectives, with which objects of some size are generally examined. Unfortunately, with objectives of a higher power than the quarter-inch, this does not seem to be so readily attainable; but as with these either very minute objects are examined in the middle of the field, or only very small portions of larger objects, it is not a very serious source of trouble, since a very slight motion of the fine adjustment serves to bring the different portions of the field successively into focus. The objective should be free from spherical aberration, a defect depending upon the lenses not being ground to the proper curves, or a distinct image is unobtainable. If the images of objects viewed under a particular objective are fringed with a border of several colours, such an objective is not properly corrected for colour—it exhibits chromatic aberration, which is a very bad fault. All objectives, apochromatics excepted, show some little colour, which in the best glasses is of a reddish-purple tint (port-wine colour), and this arises from what is known as “irrational dispersion.” It is impossible to correct the whole of the colours of the spectrum with lenses made of different kinds of glass, but by introducing into the combination a lens made of the mineral substance *fluorite*, as is done in the apochromatics, colour can be totally suppressed.

Illumination.—The microscope may be used either with natural or artificial light. In the former case we utilise the light reflected from the sky, or better, that from a white cloud. Direct sunlight must never be used. In choosing a room for microscopical work, one with a window looking towards the north should be secured if possible, for here we are never troubled with direct sunlight. In case we are unable to obtain a room with a northern aspect, then the window should be provided with a roller blind, to shut out the direct rays of the sun. The best material for such a blind is tracing-linen. This, when the sun shines on it, gives a bright white light well adapted for microscopical observation.

A small flat-flamed paraffin lamp is one of the most convenient and readily obtainable sources of artificial light for microscopic work after dark. It should be provided with a shade, so as to protect the eyes of the observer from the glare of the direct light. A chimney of a light-blue tint, or a piece of glass of the same colour placed between the light and the mirror, takes off the yellow glare of the lamp-light. The light should be sufficiently modified by introducing discs of optically worked tinted glass between the mirror and condenser, so as not to be glaring, for nothing fatigues the eyes so much as a dazzling field.

Exceedingly convenient lamps for microscopical work are supplied by the opticians. In these the chimney is made of metal, and as it has only an

aperture in front to permit the exit of light, the remainder of the room is kept in comparative darkness. In front of this aperture a slide is provided, into which fits loosely an ordinary glass slip. This is an exceedingly convenient arrangement, since, if the glass breaks owing to the heat, it can be immediately replaced by another. The light from such a lamp may be modified at will by inserting a slip of blue glass of the size of a cover-glass. Such slips, of varying depths of tint, should be kept at hand for this purpose.

Diaphragms.—When using the higher powers, a diaphragm perforated with a small aperture must be employed. This should be slightly less in diameter than the front lens of the objective, but the field of view should be illuminated up to its border. If too small a diaphragm is used, only the central part of the field will be illuminated.

Drawing Apparatus.—Nothing conduces so much to produce a lasting impression on the mind as drawing objects seen under the microscope. Although any person with a competent knowledge of drawing may do this without assistance, yet, if absolutely accurate pictures are to be produced, such as will allow of comparison with one another, then one of those arrangements must be used which, while permitting the observer to see the image of the object under the microscope, also allows him to see the point of his pencil and the surface of the paper. Several such appliances may be obtained, the best known being the camera lucida.

Glass Slips.—A number of these will be required; they serve to support the object during observation under the microscope. They are thin level slips of glass, with ground edges, the size most commonly employed being three inches by one inch. A quantity of hollow-ground slips should also be provided; these are somewhat thicker, and have a circular or oval hollow trough ground on one of their sides. They are very frequently employed in brewing work.

Cover-Glasses.—These are very thin slips of glass, varying from 0.1 to 0.3 millimetre ($\frac{1}{250}$ to $\frac{3}{80}$ of an inch) in thickness. They are either cut into squares or circles of from three-eighths to three-quarters of an inch in diameter, but larger sizes are occasionally required. Cover-glasses differ much in thickness, and as “dry” or “water immersion” objectives are constructed to yield their best results with a cover-glass of a definite thickness, care should be taken to obtain glasses of the various thicknesses suitable to the different objectives the observer employs.

Micro-Chemical Reagents.—A numerous class of substances of this kind are used in microscopical manipulation; they cause certain reactions coloured or otherwise, with the various constituents of animal or vegetable tissues, by means of which these constituents can be identified. For our present purpose only two of these will be necessary: iodine solution, made by adding 0.5 gramme of iodine and 2 grammes of potassium iodide to 150 c.c. (cubic centimetres) of water, the mixture being frequently shaken until perfect solution is effected; caustic potash solution, made by dissolving 0.5 gramme of that substance in 100 c.c. of water.

Staining Fluids.—A number of these, which are generally solutions of the aniline colours, are also employed in microscopical work. The various tissues stain in different ways, or take one stain and reject others; consequently, much assistance is obtained in this way in their differentiation. One staining fluid will suffice for present purposes, and for this methylene-blue is chosen, because its solution keeps indefinitely, which is not generally the case with the rest. The stain is made by dissolving one part of the dye in 100 parts of water.

Microscopical Manipulation.—In commencing, a low-power objective is screwed on to the nose of the microscope. If the body-tube slides in a sheath, then it may be entirely removed for this purpose, care being taken that the eye-piece does not fall out during the manipulation. This is best avoided by holding the eye-piece with the index-finger of the left hand, whilst the thumb and remaining fingers grasp the tube. In case the instrument is provided with a rack-and-pinion coarse adjustment, the tube must be racked out as far as it will go, and the objective then screwed on. In the former case the tube is replaced and pushed down to within the distance of a quarter of an inch from the stage; in the latter, the tube is racked down to the same distance. The instrument is then placed before a window, the body-tube lightly inclined at an angle, and the mirror (the plane one this time, as a low power is being used) moved about until the field of view is equally illuminated. Care must be taken that the tail-piece which supports the mirror is perfectly central, and does not hang to one side or the other, as we now wish to observe by direct, and not by oblique light. If, on looking through the instrument, black spots of irregular outline are often seen in the field, these are caused by dust or dirt on one or both of the lenses of the eye-piece, and, if this be slowly rotated, the specks will be seen to move round with it. In this case the upper lens must be unscrewed, the dirt on it removed with a piece of soft linen rag or chamois leather, replaced, and looked through to see if the objects have disappeared. If not, it is the lower lens on which the dirt is lodged; this must be removed and similarly cleaned.

Interpretation of the Appearance of Objects as seen under the Microscope.—Objects observed by the unaided vision are almost invariably seen by reflected light, rarely by transmitted. In microscopical observation these conditions are reversed; here it is comparatively rare that we use direct light in the examination of objects; they are in most cases examined in a thin layer of some fluid, generally water; consequently, their appearance as seen under the microscope is entirely different from that of objects as we usually see them. For this reason it is necessary that the microscopist should familiarise himself with the appearance of objects as seen under the microscope, so that he may be able to interpret their forms correctly. In order to do this, it is best to begin by examining a few objects of known shape, first by direct, then by transmitted light. For this purpose take a piece of thin glass rod, heat about half an inch of it in the flame of a Bunsen-burner or blowpipe until softened, then draw it out quickly until a strand of glass about the

thickness of a hair is obtained. Repeat the process with a piece of thin glass tube; in this manner a fine tube the thickness of a hair will be obtained. Now place fragments of these, about an inch long, on a glass slip; block up the aperture in the stage by the proper appliance for that purpose, and examine these two objects by direct light, using the lowest power objective and keeping the microscope in a vertical position. In order to find the correct focus, the body of the instrument is pushed or racked down until the objective nearly touches the object, and, while looking down the microscope, the body is slowly raised until the object comes into view. Should the objective have been raised some considerable distance, say an inch, without the object being seen, then the correct point has been overstepped, and the process must be commenced anew. This is the best way of finding an object for a beginner; but with practice, and a correct appreciation of the distance (technically called the "working distance") which the various objectives require, the worker will be able to push the tube down until the object is found. Great care must be taken in these manipulations, or the objective may be brought in forcible contact with the object and damaged; this is especially liable to occur with a rack-and-pinion adjustment. Having caught sight of the object, the final focussing is proceeded with; this is effected by rotating the milled head of the fine adjustment first in one direction, then in the other, until the object is seen sharply defined. We now see these objects under the usual conditions of ordinary vision, that is, by reflected light, and shall have no difficulty in detecting which is the solid rod and which is the tube. Now place a drop of water on these two objects and cover them with a cover-glass, avoiding the introduction of air-bubbles. This is best effected by holding the cover-glass at an angle, bringing one of its sides into contact with the glass slip, and then slowly lowering it into position. Move the minute tube and rod so that they occupy a position at right angles to the long axis of the glass slip; leave the aperture in the stage completely open, that is, without diaphragm; place the mirror in position, and focus. A difficulty will now be experienced in detecting which is the rod and which is the tube. This may be instantly dispelled by swinging the mirror to one side, at the same time adjusting it so that there is always some light in the field. The white strip of light in the rod moves as the mirror moves, and follows the direction of its motion; thus, if the mirror be moved to the left, the strip of light in the rod will move to the left. The strip of light in the middle of the tube moves also, but in a direction opposite to that in which the mirror moves. Now place the mirror in its former central position, and note the difference in the appearance of the two objects; the rod has a single bright line running down its centre, with two dark borders; the tube has a number of alternate bright and dark lines. Touch the end of the tube with a drop of water—water will enter it by capillary attraction; stop when the tube is half filled; examine and note the difference in appearance between the portion containing water and the empty one. That portion which is filled has lost its complicated series of lines, and now looks like an empty tube. Swing

the mirror, and note the appearances under oblique illumination. Now take off the objects and dry them externally. Having also cleaned the slip and cover-glass, examine the two objects in a drop of glycerin. The rod has lost nearly the whole of its black border, and appears like a flat strip of glass. The walls of the tube also look like strips of flat glass, and the bands of white and black in its empty portion are much more strongly pronounced than they were when it was viewed in water; they are slightly more so in the part filled with water. Examine a little wheat-meal in a drop of water, and note the circular starch cells, which are seen with perfect distinctness. Now examine another portion of the meal in a drop of turpentine; the starch cells are almost invisible.

Now place a drop of wort, mucilage, or white of egg on a glass slip, and beat it up with a penknife so as to introduce bubbles of air; place on a cover-glass and examine under the microscope. Get a perfectly circular bubble in the centre of the field, and examine with central light. The bubble, when in focus, appears as a black disc with a small bright spot exactly in its centre when the light is central.¹ Slowly elevate the body of the microscope; the centre gradually becomes dim, and the bubble loses its sharp outline. After focussing again, swing the mirror to one side; the bright spot moves in a direction contrary to that of the mirror; under very oblique illumination an air-bubble appears as a black thick ring with a grey centre, having a very bright spot at its side. Now beat a drop of oil of any kind into one of the liquids mentioned above, cover, and examine. The bubble resembles to some extent an air-bubble, but its dark border is not so wide. Focus up, and notice that, as the tube rises, the centre of the oil-globule is the last to disappear. Swing the mirror to one side; the bright spot follows the motion of the mirror. The cause of these differences in the appearances in the same objects, when seen under the varying conditions of observation, is owing to the difference in their refractive power and in that of the various media in which they are being examined. The oil-globule and the air-bubble were very distinct, because each differs greatly in refractive power from the medium in which it was surrounded; oil has a much higher refractive power than the liquid medium, air a much less. Glass has a refractive power, higher than either of them, and glycerin has a power nearly as high as that of glass. Turpentine has almost the same refractive power as starch; hence starch is almost invisible when viewed in that liquid. Had we examined the glass rod in a drop of cedar-wood oil, which has exactly the refractive power of glass, it would have been perfectly invisible.

The results of these few simple observations, if carefully borne in mind, will afford considerable help in assisting the observer to the proper interpretation of the forms and nature of the objects he has to examine under the microscope.

¹ Thus the appearance of the bright spot in an air-bubble forms a convenient means of ascertaining when the light is central.

Microscopical Examination of the Starches.—We now proceed to the examination of some natural objects, and for the first of these we choose potato-starch, as it is always readily obtainable. A drop of clean water is placed on a clean glass slip by means of a glass rod, and after cutting off a slice from a well-washed potato with a sharp knife, a trace of the substance which is scraped from the cut surface is mixed with the drop of water on the slide. A clean cover-glass is then placed over the drop, which, if of the right size (and with a little practice the right quantity can generally be judged), there will be no exudation from the sides of the preparation. If there is, the superfluous liquid must be removed by absorbing it with a small piece of blotting-paper. The preparation is now placed under the microscope, and the diaphragm suitable to the objective under employment having been put into position, we proceed to focus. A number of colourless bodies, such as those represented in our illustrations of the starches (Fig. 13 *a*, p. 73), will be seen, varying much in size, the larger ones being more or less oval, the smaller ones nearly round; these are the starch granules. In order to examine them more minutely, the glass slip is moved about until a portion of the field is found on which the grains do not lie too closely together. The weaker objective is now removed and replaced by the stronger, the diaphragm changed for a smaller, and the focussing performed as before. This time, however, it will be necessary to move the microscope much more slowly upwards in order to catch sight of the object. When in focus, the larger starch grains are seen to be covered with a series of concentric rings, which show that each starch grain is built up of a series of layers. The rings are arranged excentrically around a point situated at one end of the grain, called the "hilum," which often exhibits a number of minute rifts. The smaller grains are rounder; they do not show the stratification so distinctly, and are much more centric in construction, the hilum being in the centre of the grain and the rings more circular. Besides these, compound grains will be occasionally met with; these consist of two or more starch grains, between which a dark dividing-line is generally discernible.

It sometimes happens, in examining a preparation straight away with a higher power, that no trace of the object can be found, because in that portion of the field immediately under the objective no object or portion of one happens to be present. In this case the body of the instrument must be lowered, and, while it is being drawn up to focus, the glass slip must be moved about on the stage. As soon as shadowy images are detected, the glass slip is allowed to remain at rest, and the focussing with the fine adjustment proceeded with. If there are only one or two very small objects in the preparation, it is better to find them with the lower power first, and then exchange the objective for the stronger. It is in such cases as these that the nose-piece is especially convenient.

Dirt on Objective.—Sometimes after focussing, or even moving the object about, especially when using one of the higher powers, the field of view appears hazy, and the object blurred and indistinct. This arises from the

objective having come into contact with the liquid on the glass slip. In such a case the tube must be elevated, and the adherent liquid removed from the surface of the front lens with a piece of soft linen rag or wash-leather.

Effect of Micro-Chemical Reagents on Starch.—We now proceed to observe the effect of our two reagents on the starch granules. We place a drop of the dilute caustic potash solution on the side of the cover-glass, with its border touching that of the film of fluid under the cover-glass. The potash solution will gradually penetrate under the cover-glass and come into contact with the starch grains. Looking down the tube, we soon detect the progress of the drop, for as it touches the individual granules, these, after showing their lamination much more clearly for a short time, commence to swell out, the hilum of each enlarges and becomes a mass of rifts, and, finally, each granule attains a size ten or twelve times larger than it originally had, the contents of the granules become perfectly transparent and their borders scarcely visible.

The same appearance can be brought about by the agency of heat, and when they are produced in this way the starch is said to be gelatinised. In order to observe this phenomenon, a fresh preparation is made as before, and carefully heated over a small gas-flame, moving the glass slip to and fro so as to apply the heat equally, or the glass may crack. Even with the naked eye a change is seen to take place as the heating proceeds, and when the object is examined under the microscope, the starch granules will be found to present the same swollen and modified appearance that they did when treated with potash solution. The preparation is now to be put on one side for a time, as it will be wanted again. Gelatinisation of potato-starch takes place at a temperature of about 70° C. (160° F.), but the gelatinisation temperature of the different starches varies considerably (see p. 77). An appliance has been contrived by means of which a preparation of starch can be gradually heated up to a known temperature while under observation on the stage of the microscope, and by its aid the temperature at which the various starches gelatinise has been ascertained with great exactitude.

In order to note the behaviour of starch with iodine, a fresh preparation is made, focussed under the microscope, and a drop of the iodine-potassium-iodide solution placed on the border of the cover-glass. As this penetrates and comes into contact with the starch granules, we observe that each of these turns first a pale blue; this colour then gradually deepens until the grains appear blue-black. The progress of the iodine solution through the preparation can be followed by moving the glass slip about. The preparation in which the starch has been gelatinised by heat can now be similarly treated; as the iodine reaches the almost invisible swollen starch granules, they turn a deep blue colour, and although hardly discernible before, they now stand out prominently. The starch treated with the potash solution might have been similarly treated, and a similar result obtained; but in this case a much larger quantity of iodine solution would have been required to colour them, because caustic potash combines with iodine, and it is only after the potash

solution has been saturated with iodine, and some of the free element is present in the solution, that the coloration can take place.

The general appearance and behaviour with micro-chemical reagents may be studied with the other starches commonly met with. A little wheat, rye-flour, or oatmeal may be placed in a drop of water, and each of these starches examined in turn. In order to obtain barley, malt, maize or rice starches, a grain of these substances is cut in two with a sharp penknife, a little of the white interior scraped off and examined in water. Figures of these starches will be found on pages 72 and 73.

The starch of green malt is specially interesting. When this is examined, it is found that many of the individual starch grains are corroded; a number of canals will be found running from the outside to the inside of the grains; these are caused by the action of the diastase. The same appearance may be brought about by mixing a small quantity of starch on a glass slip with a few drops of saliva, and keeping the whole at a temperature of 38°C . (100°F .) for a little time. Saliva contains the enzyme ptyalin, which acts upon starch in a manner similar to that in which diastase does. This experiment may be rendered particularly instructive if the action be allowed to proceed for a considerable time; all the granulose of the starch grains is said to be dissolved out, skeletons of starch cellulose being left, which accurately preserve the size and shape of the original starch grains.

Examination of Yeast.—Next place a minute trace of yeast on a glass slip, mix it intimately with a drop of water, and examine under a high power. The yeast-cells appear as round or slightly oval bodies, much smaller than starch grains. Treated with iodine, the contents of the cells are stained yellow, or brown when the cells are rich in glycogen; no blue colour is observed, hence they do not contain ordinary starch. If a fresh preparation be treated with caustic potash solution, the cells do not swell up; they gradually lose their granular appearance, become clear and transparent, because the potash solution dissolves the substance within the cells. Next make a fresh preparation, and while observing it under the higher power, press on the cover-glass with the point of a dissecting-needle,¹ and notice that the cells are elastic. Press still harder until some of the cells are ruptured; notice that the contents which exude are of a tough, elastic, jelly-like nature; this semi-solid substance, which fills each cell, is the protoplasm. Those cells from which the protoplasm has exuded now look like little sacks, which, on emptying, contract to a slight extent, thus showing that before being ruptured they were to a certain extent distended by internal pressure. Now run in a little of the methylene blue solution.² The contents of all those cells which are disorganised stain a deep blue; the uninjured cells remain, as before, colourless. This experiment exhibits one of the peculiar properties of proto-

¹ These are made by pushing a medium-sized needle held with a pair of pliers point foremost into a soft wooden penholder, withdrawing the needle, and then pushing it in head foremost for half an inch.

² Too much of the stain must not be used or it will kill the yeast-cells.

plasm: when alive it refuses to be stained by any of the dyes; when dead it evinces a powerful attraction for these substances.

Place a little yeast and water on a glass slip and heat it nearly to boiling temperature; afterwards add a little of the stain and examine it; all the yeast-cells now take the dye—they are dead. This behaviour of living protoplasm with reference to tinctorial substances affords an excellent method of detecting the dead yeast-cells in a sample of yeast; it is only necessary to place a little of the yeast on a glass slip, mix it with a drop of water, and then run in a small quantity of methylene blue solution, or almost any other dye, when the dead cells are readily detected by their coloured appearance. Now make a fresh preparation of yeast, and, by pressing on the cover-glass with the finger-nail, rupture some of the cells; then grind their contents between the cover-glass and glass slip. On examination a number of exceedingly minute bodies, the microsomes, will be seen. These are in a state of lively movement, which conveys the impression that we are looking at living organisms. This, however, is not the case; all very minute bodies when examined in a state of suspension in a liquid have this peculiar motion, first called attention to by Brown, and hence called after him the “Brownian movement.” When examining very minute organisms, such as bacteria, it is often difficult to distinguish the living organisms from the minute particles of dead matter; but, if careful attention be paid to the nature of the movement, the two objects may generally be differentiated. In the Brownian movement the motion is more localised, the objects rotating in sinuous lines about an imaginary centre; with bacteria the movement is a more independent one, they move about the field in all directions.

The Hanging-Drop Method.—We will now proceed to examine yeast by another method, using one of the glass slips with a circular hollow ground in it. After being cleaned, paint, with a small camel-hair pencil, a ring of vaseline about the eighth of an inch wide round the border of the hollow. Place about 10 c.c. of wort in a test-tube, or, if wort is not at hand, of a 10 per cent. solution of cane-sugar; mix intimately with this a piece of stiff yeast about the size of a pin’s head. Next place a minute drop of the mixture in the centre of a clean cover-glass,¹ turn the glass upside down and breathe into the hollow of the glass slip so as to slightly moisten its surface, and lay on it the cover-glass so that the drop occupies the centre of the hollow; press down gently. The vaseline ring makes an air-tight seal, the drop is now in a closed chamber, and may be kept for weeks or months without suffering loss from evaporation. Place under the microscope, and if the result is successful, not more than two or three yeast-cells will be seen in the drop. If more than three cells are present in the drop, it is best to begin again, and dilute the mixture in the test-tube with more wort or sugar-water until a drop is obtained containing the right number. Make a rough drawing of the appearance of the yeast-cells, and keep the preparation under the

¹ This is best done with one of the steel goose-quill pens which are sold for drawing maps.

microscope, observing it from time to time. Note how the yeast-cells first throw out buds, how these increase in size, and finally become detached from the parent cell. The preparation, after being examined throughout the day, can be placed on one side and examined occasionally during several subsequent days. In a few days the two or three cells will have multiplied to hundreds. It is obvious that by using for the preparation a liquid entirely free from other organisms, and taking care that there is only one yeast-cell present in the drop, we are able, in this way, to secure a pure culture of yeast from a single cell. This method also affords an exceedingly convenient way of studying the growth of many minute organisms. Growths of the various mould-fungi made in this manner are described in the chapter devoted to those organisms.

Objects often met with in Polluted Water.—When water is polluted with refuse from human habitations, portions of articles of food, clothing, &c., are generally to be found in it. It is well, therefore, for the microscopist to make himself acquainted with the appearance of such objects as seen under the microscope. For this purpose, shreds of cotton, woollen, and silken clothing should be teased out with a pair of needles in a drop of water, examined under the microscope, and their general appearance noted. These will almost invariably be coloured, since the majority of materials made from these substances are dyed. Portions of lean meat should be similarly treated—here we note the appearance of muscular fibre; also portions of vegetable tissues, such as leaves and stalks of cabbage, the husks of corn, &c. Human hair and the hair of animals is also frequently found in water, portions of the feathers of birds, epithelium scales; the last may always be seen in a drop of saliva.

Microscopical Examination of Bacteria.—As these are the smallest organisms known, we require for their examination the best and most powerful objectives and the most favourable conditions of illumination. Though their presence may be readily detected with an eighth or a tenth of an inch dry objective, a homogeneous immersion objective becomes a necessity if their complete investigation is to be undertaken. Water immersion objectives may be employed, but, as stated before, the twelfth of an inch oil immersion is the best objective for this class of work.

Methods of Obtaining Bacteria for Examination.—As bacteria, or their germs, are omnipresent, specimens of these organisms are very easily obtained for examination. It is only necessary to boil a potato, cut it into slices, expose the freshly-cut surfaces to the air for a few hours, and then place the slices in a moist chamber, which latter may be extemporised with an ordinary basin and a dinner-plate, two or three sheets of filter-paper (cut into a circular shape and moistened with water) being placed on the plate. The slices of potato are laid on the moist filter-paper, covered up, and allowed to stand at the ordinary room temperature for several days, being examined at intervals. During the time they were exposed to the air, germs and bacteria in greater or less numbers will have fallen upon the slices; and since the

conditions mentioned above are favourable for their growth and development, the organisms and germs will in a few days have germinated, multiplied, and formed patches which are technically called "colonies," and which appear as small, whitish, and occasionally coloured, gelatinous masses. A small portion of any one of these colonies may be removed by means of a platinum wire, mixed intimately with a drop of water on a glass slip, covered with a thin cover-glass, and examined under the microscope in the usual manner. Bacteria may also be obtained in abundance by making an infusion of lean meat and allowing it to stand in a warm place for from twenty-four to forty-eight hours, when it will be found to be literally swarming with bacteria. Vegetable infusions made from hay, turnips, peas, &c., when similarly treated, yield bacterial organisms in immense numbers. If a small quantity of malt or barley be covered with distilled water and allowed to stand for a week, the liquid will be found to swarm with bacteria and yeasts. It is only necessary to take a small drop of such an infusion, place it on a glass slip, put on a cover-glass, and examine it under the microscope, when large numbers of these organisms will be seen of various sizes and shapes, some quiescent, some in a lively state of motion.

It is interesting to examine a culture which contains only one single species of bacteria. The *Bacillus subtilis*, or hay-bacillus, owing to the resistance of its spores to boiling water, readily permits this to be effected. In order to obtain a pure culture of this organism, dry hay is soaked in the smallest possible quantity of water and allowed to stand at a temperature of about 36° C. (97° F.) for a few hours, the infusion so obtained is poured off without filtering, and diluted with water until it has a specific gravity of 1.004. It is then placed in a flask, and after a plug of cotton-wool has been inserted in the neck of the flask, gently boiled for an hour, cooled, and kept at 36° C. (97° F.) until a film of bacteria appears on its surface. This will generally be the case in twenty-four or thirty-six hours.

We take a minute piece of this pellicle off by means of a wire, and place it on a glass slip, together with a minute drop of the liquid; place on a cover-glass, and examine with the 1-8-inch dry objective. The pellicle is seen to be formed of long wavy threads lying parallel to one another, each of which is divided into a number of segments, each segment being a separate individual. The segmentation can be rendered more distinct by running a little iodine solution under the cover-glass, which stains the organisms a brownish-yellow colour. The threads are held together in an immovable position by a mass of invisible jelly, produced by the swelling of the slimy coats of the organisms. Bacteria, in this state, are said to be in the "zooglear" condition.

Being certain that we have the object in the field, we proceed to its examination with the immersion objective. If the condenser is not already in position, it must be so placed, using one of the smallest diaphragms and the plane mirror. The objective having been screwed in its place, a small

drop of the cedar-wood oil,¹ which was supplied with the lens, is placed in the middle of the cover-glass, the microscope pushed or racked down until the nose of the objective dips into the oil-drop and nearly touches the cover-glass. Focussing is proceeded with as usual, taking care to move the microscope body very slowly upwards. It is here that the benefit of a rack-and-pinion movement is experienced. The condenser must now be focussed. If we are using daylight, we move the mirror about until a shadowy glimpse is caught of one of the window bars, then the condenser is moved slowly up and down until the bar is seen distinctly in focus. The mirror is then moved until the field is equally illuminated. When working by lamplight, the lamp is adjusted so that the edge of the flame is turned towards the microscope, and the condenser focussed until the image of the flame appears clearly defined. The best conditions of illumination are when a perfectly flat superficies of achromatic light is in the focus of the objective at the same time that the object itself is in focus, and the diameter of the section of the cone of light which enters the objective is about three-fourths the diameter of the front lens of the objective. To obtain the very best possible conditions of observation, the condenser requires as much care in its construction and use as the objective itself.

We now proceed to examine the hay bacilli again, first using the lower-power eye-piece and then proceeding to the higher-power one. It must be remembered that an oil immersion will bear a much higher power, or, as it is often called, a much deeper eye-piece, than a dry objective. The bodies of the individual bacilli are seen to be composed of a colourless, homogeneous, somewhat highly refractive substance, in which, even with the highest powers at our command, no trace of structure or of a nucleus can be detected.

We then make a hanging-drop preparation, taking for this a minute portion of the pellicle and a very small drop of the liquid. By carefully noting the appearance of such a preparation (which is best effected by drawing it with the aid of camera lucida), and by keeping the preparation for some time under observation, we are able to observe the process by which the bacilli grow. We see that each segment gradually increases to a certain length, whilst preserving the same thickness; then a partition-wall gradually forms which divides the organism into two. It is owing to this growing in length that the bacilli come to be arranged in lines. After the lapse of six or eight hours the nutriment in the drop becomes exhausted; the bacilli then cease to grow, and begin to form spores. When this commences, bright spots make their appearance in many of the bacilli, which gradually increase in size, until at last each presents the appearance of an ellipsoidal, bright, strongly refractive body with sharp outlines; these are the "spores." As the process is proceeding the remaining contents of the organism gradually disappear, the cell-wall finally disintegrates, and the spores are set free. These, when introduced into a fresh nutritive medium, soon commence to germinate, especially if

¹ Care must be taken that the fluid which oozes from between the slip- and cover-glass does not become mixed with the cedar-wood oil, or, when examining an object mounted in Canada balsam, that the oil does not get contaminated with the balsam.

they have been boiled for a few minutes; they then commence to germinate in two or three hours. The envelope of the spore ruptures on one side; from this protrudes a bud, which gradually enlarges in length to form a bacillus, which divides by segmentation for the first time in about twelve hours. The organisms do not immediately enter into the zooglycal state, but pass through what is termed the "swarming stage." In this, pairs or chains of individuals are seen moving about in a very lively manner. They are propelled by delicate hair-like appendages at each end, technically called "flagella," which can be readily seen when the organism has been stained in a particular manner. The swarming bacteria, after living in the body of the liquid for some time, ascend to its surface and enter into the zooglycal condition. This bacillus has been studied by Adrian Brown¹ in relation to wort and beer, who finds that it cannot grow in normal wort or beer owing to the natural acid reaction of these liquids. Its chief interest resides in the extreme resistance of its spores to heat.

Bacteria in the swarming stage may be very conveniently examined by the hanging-drop method; in this form of preparation the organisms are under conditions closely resembling those in which they naturally exist. At the sides of the drop they will be in a more or less quiescent state, and permit their size and shape to be readily observed; whilst in the middle of the drop they have more room for their evolutions, and are uninfluenced by the capillarity conditions which exist at its border. By examining the organisms in the upper layer of the middle of the drop, which are sufficiently near the surface to be within the range of focus of the object-glass, we are able to form some opinion of their behaviour under conditions closely approximating to those in which they naturally exist.

The Stained Cover-Glass Preparation.—Bacteria are much more easily seen when stained, and in this condition, being dead, we are no longer inconvenienced by their movements. In making such a preparation, a small drop of the liquid containing the bacteria is placed on a thin cover-glass by means of a sterilised platinum wire, and spread out as much as possible with the wire into a thin layer.² It is now left until the liquid has evaporated and the surface appears dry. The bacteria are now to be "fixed," or they would be washed away during the subsequent staining and rinsing. This is effected by exposing the cover-glass and its adherent bacteria to a temperature of 120° to 130° C. (248° to 266° F.) for five minutes in a hot-air oven. In this way the mucilaginous coats of the bacteria become adherent to the cover-glass. The process of fixing may be readily executed in the following manner: The cover-glass is taken up with a pair of tweezers, the surface to which the bacteria are attached being kept upwards; the cover-glass is then passed three

¹ *Journal Fed. Inst. Brewing*, 1895, 1, 423.

² In order to make a successful preparation in this way, it is necessary that the cover-glass be absolutely clean, or the drop will not spread evenly. The cover-glasses, after having been washed with water, should be heated in a mixture of strong sulphuric acid and potassium bichromate, afterwards well rinsed in distilled water, and then kept in alcohol until required for use.

times through the flame of a Bunsen-burner or of a spirit-lamp. The heating must be carefully performed; if insufficient, the bacteria will be afterwards washed away; if overdone, the bacteria refuse to take the stain well. The happy medium is obtained by moving the hand vertically in a circle a foot in diameter; the time taken to complete the circle should be one second. When traversing the lower portion of the circle the preparation is momentarily introduced into the flame, but care must be taken that no halt takes place here, or the preparation will be overheated. The movement in describing the three circles must be a constant and uninterrupted one, so that the preparation is exposed momentarily thrice to the heating influence of the flame. When the cover-glass has become cold, its upper surface is covered with a large drop of staining solution (see below), which is allowed to remain there for several minutes. In case the bacteria do not take the stain freely, the preparation may be held over a small flame until the drop of staining liquid steams. The cover-glass is now gently rinsed with clean water to remove the staining liquid (which is best effected by a gentle stream of water blown from a pipette), then placed with the side on which the bacteria are fixed downwards on a glass slip, its upper surface dried with blotting-paper and examined under the microscope. When viewing stained preparations, the diaphragm of the condenser is opened to its full extent, so as to admit as much light as possible.

Conversion in a Permanent Preparation.—The preparation obtained in the foregoing manner can be easily converted into a permanent one. For this purpose the cover-glass is removed from the slip and allowed to dry. A drop of a mixture of equal parts of Canada balsam and xylene is placed in the middle of a clean glass-slip, the cover-glass taken up with a pair of tweezers (care being taken that the side to which the bacteria are attached is downwards) and slowly and gently dropped down on the drop of balsam, in such a manner that no bubbles of air are included. A little gentle pressure applied to the cover-glass will cause the balsam to spread out to the margin of the cover-glass, and, if the proper quantity of balsam has been taken, it will be just sufficient to do this and no more. If, however, as often happens, rather too much balsam has been used, the excess must be carefully removed by means of a piece of linen rag moistened with benzene or xylene, or, when the cover-glass is afterwards covered with cedar-oil in order that it may be viewed with the immersion lens, the Canada balsam will dissolve in the oil and alter its refractive index, and it will also soil the lens. In a few days the balsam dries and secures the cover-glass; after a few weeks the balsam becomes thoroughly hardened, and in this way a permanent preparation is secured. All preparations coloured with aniline colours should be carefully kept excluded from the light, otherwise they are apt to fade.

In order to identify the different species of bacteria, it is necessary to have recourse to certain methods which have for their object the isolation of the different species; a description of these methods forms the subject of the following section.

BACTERIOLOGICAL METHODS

In order to identify and investigate the nature and properties of the various bacterial organisms, it is necessary to first isolate them. This having been done, it then becomes possible to study the behaviour of each species under certain specified methods of treatment, by means of which their identification is effected. The effects produced on various media by different species can also be studied; for instance, we may endeavour to ascertain if a particular organism turns wort or beer sour, if it renders either liquid turbid or ropy, or if it produces a pronounced flavour in one or both of these liquids.

Of the various methods for effecting the isolation of the individual bacteria which have been proposed from time to time, that invented by Dr. Koch is the one almost universally employed. The principle of his process is to widely separate the individual bacteria from one another in a solid nutrient medium. This is spread out in a thin layer on a plate of glass, and then placed under conditions favourable to the growth and development of bacterial organisms. In this way each individual bacterium increases and multiplies; in other words, it forms a colony, the members of which are retained in position, and not permitted to wander about, by the solid nature of the culture medium. In the course of a few days the colonies become sufficiently large to be detected by the naked eye; they can then be examined in various ways.

Sterilisation.—In carrying on bacteriological work, it is not only necessary that all the utensils and apparatus employed should be in a state of absolute cleanliness, but that they should be also sterile, that is, completely freed from living organisms. The media made use of in such work must also be rendered sterile. No pains should be spared to secure these conditions, otherwise the results of any investigation in which they are not rigidly adhered to will be valueless.

Several methods are in use for securing sterility of the apparatus. Many chemical substances are known, such as corrosive sublimate, salicylic acid, sulphurous acid, &c., which act as powerful bactericides, and these are occasionally used for the object in view; but the agency chiefly relied upon is heat, applied in one form or another. Heat applied in the form of steam saturated with aqueous vapour is a much more powerful steriliser than what is technically known as “dry heat,” such as that obtained by employing dry air or superheated steam. No organisms or their germs are able to withstand a prolonged exposure to dry air heated to 150° C. (300° F.), and but one germ, the spore of the *Bacillus subtilis*, is known which can survive prolonged boiling in water or lengthened exposure to the heat of steam saturated with moisture having a similar temperature. Liquid culture media, such as wort, grape-juice, &c., are generally boiled in order to effect their sterilisation.

Sterilising by the Direct Heat of a Flame.—This is a very convenient way of sterilising small articles, such as platinum wires, microscopical slips,

cover-glasses, tweezers, &c. The articles are simply passed through a Bunsen flame or that of a spirit-lamp; in this way any organisms or germs present on such articles are destroyed. In case of emergency, quite large articles, such as cultivation-plates, Petri-dishes, &c., may be similarly treated, but great care must be taken with glass articles, more especially with the larger ones, as they easily break to pieces on heating.

The Hot-Air Oven.—Much use is made of this form of apparatus for the sterilisation of such articles as glass plates, test-tubes, Petri-dishes, cotton-wool, &c., which are able to withstand the comparatively high temperature required without injury.

An apparatus of this kind usually consists of a rectangular box of sheet metal with double walls, having a door in front such as is seen in Fig. 18. In its roof are two apertures—the one intended for the insertion of a thermometer; the other for the thermo-regulator by means of which the oven is maintained at a definite temperature. The oven is heated by a Bunsen or other form of gas-burner, the gas supply to which passes through the thermo-regulator. Shelves are provided for supporting the various articles, and trays of wire-gauze for holding such small articles as test-tubes, &c. When a number of glass plates, intended for plate-cultivations, are to be sterilised, they are usually placed in a sheet-metal box (Fig. 23, p. 237) provided with a lid, which, after being filled with plates, is placed in the sterilising oven, the lid of the box serving to protect the plates from subsequent contamination. After the oven has been filled with the articles to be sterilised, the gas is turned on, then the thermo-regulator and the sliding door in the roof of the oven manipulated until a constant temperature of 150°C . (300°F .) is indicated by the thermometer. It is maintained at this degree for two or three hours, then the gas is turned off, the oven allowed to cool down to the room temperature, and the articles it contains removed. If the oven door is opened whilst the temperature inside is still high, there is great danger of the glass articles cracking.

FIG. 18.—Hot-Air Oven.

The Thermo-regulator.—There are several forms of this apparatus; one of these is shown in the accompanying illustration, Fig. 19. It consists of a glass tube divided into two chambers, *a* and *b*, by a partition, which is perforated by a central aperture, to which is fused a thin glass tube reaching nearly to the bottom of chamber *b*. In the upper chamber a side tube, *c*, is inserted; a perforated cork closes the upper part of the apparatus, and through this passes the bent tube, *d*, which can be raised or lowered within the chamber *a* as circumstances demand. A small quantity of mercury is introduced into the

chamber *b*. The apparatus is inserted into the oven through the aperture specially provided for that purpose; one extremity of an indiarubber tube is attached to the tube *c*, the other to the burner underneath the apparatus. Another indiarubber tube coming from the gas supply is attached to the tube *d*. The action of the apparatus is as follows: When the air in the oven becomes heated, a portion of its heat is communicated to the air in the chamber *b*, causing it to expand. In doing so the mercury is driven up into the tube

FIG. 19.

in *a*. When this has risen to a certain height it covers the end of the tube *d*, and gradually cuts off the principal supply of gas, so that at last no gas can pass to the flame except through a minute opening in the tube *d*. As a consequence, the flame is nearly extinguished. The temperature in the oven then begins to lessen, the air in the bulb to contract, the mercurial column lowers, and the orifice at the bottom of *d* is once more opened, and gas admitted to the flame. After a time a sort of equilibrium becomes established between the amount of gas necessary to keep up the heat required, and then just sufficient gas passes through the regulator to maintain the oven at the required temperature. It is obvious that by adjusting the quantity of mercury in the bulb, and regulating the position of the tube *d*, any required temperature within certain limits can be steadily maintained.

Steam-Steriliser.—Culture media, especially those containing gelatin, are generally sterilised by the agency of moist steam. The apparatus used for this purpose consists of a tall cylindrical vessel constructed of sheet metal clothed with felt, which can be closed with a lid pierced by several apertures, one of which is intended for the insertion of a thermometer, the others for the escape of steam, Fig. 20. A removable vessel (Fig. 21) serves to hold the different articles which are to be sterilised. A water-gauge indicates the level of the water in the apparatus. When about to be used, water is placed in the bottom of the vessel to a depth of three or four inches, the basket with the various articles inserted, the cover placed on, and a Bunsen-burner or other source of heat placed underneath. The water is kept boiling for two or three hours at such a rate that there is a free issue of steam through the orifices in the lid; the apparatus is then allowed to cool, and the basket removed. The water-gauge must be examined from time to time, to see that all the water does not boil away; should it get low, the vessel is replenished with fresh boiling water.

The Autoclave.—In this apparatus the sterilisation is effected through the agency of steam under pressure, in which condition it possesses a higher temperature than steam existing at the ordinary atmospheric pressure. Steam when used in this way exercises a much more powerful bactericidal action than when its temperature is only that of boiling water; for instance, Globig found that certain bacteria, which survived a three hours' exposure to steam

at the temperature of 100° C. (212° F.), readily succumbed to steam at a temperature of from 110° to 120° C. (230° to 248° F.) in an autoclave. This

FIG. 20.—Koch's Steam-Steriliser.

FIG. 21.—Basket for Steam-Steriliser.

apparatus, which is illustrated in Fig. 22, resembles the ordinary steam-steriliser in shape, but since its walls have to bear considerable internal pressure, they are made correspondingly strong. The lid is made to screw down steam-tight, and is provided with a safety-valve and a pressure gauge. A removable shelf-holder serves to carry the articles to be sterilised. When used, water is placed in the bottom of the vessel to a depth of a few inches, the shelf-holder with its articles introduced, the lid screwed down, and heat applied from below by means of a large Bunsen-burner. Since water always boils at a certain temperature for a particular pressure, the heat of the interior is ascertained from the indications of the pressure gauge. Thus, when a pressure of 14.6 lb. on the square inch is indicated, the temperature will be 122° C. (252° F.). By a little regulation of the gas supply a point is soon found where the required pressure and corresponding temperature are constantly maintained. The apparatus is then kept at this temperature for one or two hours, when the source of heat is removed, the steriliser allowed to cool, and the shelf-holder removed. The safety-valve is generally loaded

FIG. 22.—Autoclave.

to a slightly higher pressure than that required in the interior of the chamber, consequently there is neither escape of steam nor loss of water. This form of apparatus is useful for the bacteriological work connected with brewing, when samples of beer have to be sterilised. If such samples are treated in the ordinary steam-steriliser a loss of alcohol takes place; by conducting the operation in an autoclave this is avoided.

Preparation of the Solid-Culture Medium.—This is prepared as follows: One pound of fresh lean beef, finely divided by chopping or running through a mincing-machine, is added to a litre of cold distilled water, and well stirred in. The mixture is allowed to stand for twenty-four hours in a cool place, an occasional stir being given, after which it is poured into a clean linen cloth placed over a large glass funnel, and the meat-juice strained off, the pulpy mass in the cloth being squeezed until a litre of meat-juice is obtained. The following substances are then added to it—100 grammes of gelatin, 10 grammes of peptone in powder, and 5 grammes of sodium chloride. The mixture, after being allowed to stand until the gelatin has swollen, is gently warmed until this substance has dissolved. Meat-juice is always slightly acid, because it contains a small quantity of lactic acid; and since bacteria flourish best in a neutral or slightly alkaline medium, the meat-juice is carefully neutralised with sodium carbonate solution until it is just neutral or faintly alkaline to litmus-paper. This neutralisation of the medium has a most important influence on the number of bacteria which afterwards develop in it. Reinsch, when experimenting with river-water, found that by employing un-neutralised gelatin meat-broth only 475 colonies per 0.5 c.c. of the water were formed; when 0.01008 gramme sodium carbonate has been added to 10 c.c. of the same medium, 2976 were formed; whilst the addition of 0.3024 gramme entirely suppressed the development of the colonies. He recommends, when experiments of a delicate nature are to be carried out, that just sufficient solution of caustic soda should be added to the medium (to which a little phenolphthalein solution has been added) to turn it pink. The necessary quantity of alkali is found by titrating an aliquot part of the medium with dilute caustic soda solution, throwing this away and then adding the calculated quantity of soda solution requisite to bring the bulk of the medium to the same state of neutrality as the aliquot part. The whole mixture is then placed in a large flask, the white of an egg is well beaten up, added, thoroughly mixed in by shaking, the flask and its contents placed in the sterilising apparatus, and exposed to a temperature of 100° C. (212° F.) for an hour. The object of the addition of the white of egg is to promote clarification.

The hot gelatin meat-broth is then filtered through fine filtering-paper, when it should run off as a perfectly bright fluid. As it rapidly solidifies on cooling, the filtration must either be conducted in a hot chamber, or the funnel which contains the filter-paper surrounded by a hot-water jacket; the operation may be performed inside the steam-steriliser. As it passes through the filter the gelatin meat-broth is caught in quantities of about 10 c.c., either

in small flasks or in test-tubes, care being taken that it does not soil the neck of the flask or the upper portion of the test-tube. Plugs of cotton-wool are inserted into the mouths of the flasks or the test-tubes, to prevent the access of germs from the atmosphere. The tubes or flasks are then placed in the steriliser, the water in it brought to the boil, and maintained at that temperature for a quarter of an hour, this treatment being repeated every day for three days in succession, the object of this procedure being to secure effectual sterilisation. It has been shown that the spores of bacteria are much more resistant to the sterilising effect of heat than the bacteria themselves, therefore a period is allowed between the consecutive heatings to afford any

FIG. 23.—Plate-Box.

FIG. 24.—Levelling Apparatus.

spores which may be present an opportunity of developing into the less resistant stage of bacteria.

This method, which is called “intermittent” sterilisation, is of great use in the technical application of the process to such purposes as the preservation of meat in hermetically closed tins, for in this way the over-cooking of the meat, which took place under the older methods of sterilisation, is to a great extent avoided.

The gelatin meat-broth tubes are then kept for a few days to see if the sterilisation has been complete. If such has been the case, the jelly will remain perfectly bright and clear; should signs of turbidity appear in any of the tubes these must be rejected, for they are evidently still contaminated with living organisms.

Preparation of the Cultivation-Plate.—The actual cultivation of the bacteria is performed on glass plates, hence the name “plate-culture.” For this purpose plates of ordinary glass are used, of such a size as can be conveniently examined under the microscope, their breadth being determined by the distance between the centre of the objective and the pillar which supports the body of the instrument, and they may be about twice as long as they are wide. Three inches by five is a size frequently used; the ordinary quarter-plates used in photography are also of a handy size for this purpose.

The plates are first thoroughly cleaned, and then sterilised by exposing them for an hour to a heat of 150°C in the hot-air oven (Fig. 18, p. 233). During this process the glass plates are enclosed in the covered box of sheet metal (Fig. 23).

In spreading the gelatin meat-broth on the glass plates a levelling apparatus,

such as that shown in Fig. 24, is generally used. This consists of a wooden tripod stand, the feet of which are formed of three screws by means of which the apparatus can be accurately levelled. The shallow glass tray, shown on the top of the glass plate in the figure, is filled with ice and water and placed on the tripod stand; this is covered with the glass plate on which the culture-plate is to rest; and on this the bell-glass, for covering the cultivation-plate and protecting it from stray germs.

When the apparatus is to be used, the tray is filled with water and ice, covered with the large plate of glass, and carefully levelled by adjusting the screws until the small spirit-level indicates that the apparatus is level. These

FIG. 25.—Moist Chamber.

FIG. 26.—Support for Cultures.

arrangements having been made, a sterilised glass plate is removed from the box and carefully placed on the glass plate; in handling it, more contact with the fingers than is absolutely necessary should be avoided. It is then covered with the bell-glass.

Several of the gelatin meat-broth tubes or flasks are heated in the water-bath until their contents have become fluid, care being taken that the heat does not rise beyond 35° C. (95° F.), or some of the bacteria might be injured; the plug is carefully removed, and held between the fingers of the right hand in such a manner that that portion of it which enters the test-tube is not soiled by the fingers. If, for instance, we wish to investigate the organisms which have developed in meat infusion, we take out a minute drop of this by means of a platinum loop,¹ and stir it into the gelatin meat-broth contained in one of the tubes (the medium should measure approximately 10 c.c.), insert the plug, and, by a rolling and twisting motion applied to the tube, mix the infusion intimately with the medium. The plug is once more removed, the upper portion of the neck of the tube passed several times through the flame of a Bunsen-burner or of a spirit-lamp, so that any adherent organisms may be destroyed. When the neck of the tube is sufficiently cool its contents are poured out, at one sweep, on to the middle of a cultivation-plate, which is placed in position on the levelling apparatus, and spread out into a thin layer with the assistance of the neck of the test-tube. The film must not be allowed to approach the edges of the plate within a distance of half an inch, as this portion may have been contaminated by the fingers during the previous manipulation. The glass cover is placed over the cultivation-plate, and the whole allowed to remain until the gelatin film has become solid; this, under the cooling influence

¹ Made out of a piece of platinum wire about one-fortieth of an inch in thickness, one end of which is fused into a glass tube, the other end twisted into a loop about one-sixteenth of an inch in diameter.

of the ice-cold water, takes place in one or two minutes. When solidification has taken place, the cultivation-plate is removed to the moist chamber.

The Moist Chamber.—This, which usually consists of two glass dishes, is represented in the illustration (Fig. 25) on p. 238. The lower dish has, as a rule, a diameter of about 8 inches and a depth of 4 inches. The other dish is slightly wider, and is used for the cover. The dishes are first thoroughly cleaned by washing, then smeared inside with glycerin, which will retain any bacteria which may fall upon them. A layer of filter-paper, moistened with thirty or forty drops of a saturated solution of mercuric chloride (corrosive sublimate, a powerful bactericide, and also a most poisonous substance), is placed in the bottom of the narrower dish. The water contained in this solution serves to keep the air in the apparatus saturated with moisture. A little stand, which will hold several culture-plates, is often employed in the apparatus, so that several cultivations may be simultaneously carried on in it (Fig. 26). It is constructed of glass plates, to the extremities of the longer sides of which strips of plate-glass about three-eighths of an inch in thickness, half-an-inch in breadth, and of the same length as the width of the culture-plate, are affixed either with sealing-wax or mineral glue. When a number of these are superimposed they form, as it were, the shelves of a small open cupboard, on which the cultivation-plates can be placed. A slip of paper, bearing a reference number or letter, is placed under each cultivation-plate, so that the plate can be afterwards identified. The moist chamber containing the cultivation-plates is then placed in the incubator.

The Incubator.—This consists of a vessel of sheet metal (Fig. 27), generally made of copper, having double walls, and provided with a door in front; the space between the walls is filled with water. The apparatus is kept at the required temperature by means of one or more gas-flames placed underneath it, the supply of gas to which is controlled by a thermo-regulator. In plate-cultivation experiments this is so adjusted that there is steadily maintained within the incubator a temperature of 20° C. (68° F.), as indicated by the thermometer, the bulb of which is inside the apparatus.

The moist chamber and its contents are introduced into the incubator, the door closed, and the whole left for three or four days, the culture-plates being occasionally examined. At the end of two or three days the individual colonies will have become visible to the naked eye, and can be further

FIG. 27.—Incubating Chamber.

examined by the aid of a hand magnifying-glass. They will differ considerably in appearance, since the meat infusion would be sure to contain numerous forms of bacteria. Some of the colonies will appear as white slimy-looking spots resting on the surface of the gelatin, others will have spread more considerably amongst the surrounding gelatin, some may have an iridescent appearance, others may be brilliantly coloured. Many of the colonies will be found to have left the gelatin film intact, others to have liquefied it, and various degrees of liquefaction may be observed amongst the different colonies.

Observation of the Colonies under the Microscope.—For this purpose, the cultivation-plate is placed on the stage of the microscope, and viewed under a power of about 100 diameters. Some of the colonies will have developed inside the gelatin film, and these will appear as dark round bodies. Others will have vegetated on the surface of the film, or have reached its surface in growing. Remarkable differences in the form, and especially in the borders, of the colonies will be observed. Some may be surrounded by a well-defined line, some by a notched and indented one, some are bordered by projections having the appearance of locks of hair, some throw out hair-like shoots, whilst in others the margins are, as it were, beset with fine prickles. These differences in appearance show that the bacteria belong to different species.

Observation of the Individual Bacteria forming the Colonies.—The bacteria may be further examined by removing a portion of the colony with a straight platinum wire attached to a glass handle, which, just before being used, is passed through the flame of a Bunsen-burner, in order to destroy any adherent bacteria. This process is performed with the culture-plate under the microscope, the particular colony being kept in view under a low magnifying power. The bacteria adhering to the platinum wire are now mixed with a small drop of water on a cover-glass; this is then placed on a glass slip and the bacteria examined either in the ordinary manner, or by the hanging-drop method (p. 226); afterwards it can be made into a permanent preparation (p. 231).

"Impression Preparations."—These are easily made, and serve for keeping a permanent record of the forms of colonies. In order to obtain an "impression preparation," a perfectly clean cover-glass is laid over the colony of which it is wished to obtain a permanent record, allowed to remain in contact with it for a moment, and then removed from the surface of the gelatin with a pair of tweezers. By this means an impression is obtained of the colony. The preparation is now allowed to become perfectly dry, and is then stained and mounted in the same manner as a cover-glass preparation. Not only can such a preparation be examined with low powers for the shape of the colony, but the highest magnifying powers may be applied so as to examine the individual bacteria. Young colonies lend themselves more readily to treatment in this way than older ones. The method is not well adapted for colonies where a large amount of liquefaction of the gelatin has taken place; in order

to make successful impression preparations of the liquefying bacteria, they must be made while the colonies are quite young.

Substitutes for the Cultivation-Plate of Koch.—Petri replaces the plate by small dishes (Fig. 28), one of which is slightly larger and fits over the other. The dishes are about four inches in diameter and half an inch high. After they have been sterilised, the warm infected gelatin meat-broth is run into the lower dish, and spread over the bottom of the dish in a thin layer; the upper dish, which forms the cover, is put in its place, and the apparatus simply left to itself until the gelatin has solidified, no artificial cooling being required. It is usual after the gelatin has set to invert the dish so that it rests in the incubator on its cover. When the colonies are formed, they can be examined under the microscope, and preparations made from them in exactly the same manner as when a cultivation-plate is employed. Esmarch has proposed another modification, in which a small quantity of the infected gelatin meat-broth is run into a sterilised test-tube, a sterilised plug of cotton-wool inserted, and, by a gentle rolling motion, the gelatin is spread in an even layer on the inside of the tube, the tube meanwhile being held under a stream of cold water, care being taken that the gelatin does not touch the plug. The colonies develop on the film in the inside of the tube, and can be counted, examined under the microscope, taken out with a bent platinum wire, and preparations made from them. This modification obviously does not permit impression preparations being taken from the film. G. H. Morris slightly extends this method by running the infected material into a globular sterilised flask, inserting a plug of sterilised cotton-wool, and distributing the gelatin in a thin layer by rotating the flask.

FIG. 28.
Petri-Dishes.

Cultivation of Anaërobic Organisms.—Organisms of this nature, such as the butyric acid ferment, which cannot live in the presence of oxygen, must be either cultivated in an atmosphere deprived of this gas, or in one of an indifferent gas such as hydrogen. One of the simplest methods for performing a culture of this kind is to infect the contents of a gelatin meat-broth test-tube, plugged with cotton-wool, in the ordinary way, and insert this into a much larger test-tube, which contains a small quantity of an alkaline solution of pyrogallol, the larger tube being closed with an indiarubber cork. As alkaline pyrogallol solution rapidly absorbs oxygen, the enclosed air is entirely deprived of this gas in a very short time.

Bacteriological Examination of Water.—A sample of water may be examined bacteriologically, either by Koch's cultivation-plate method, or by that in which Petri-dishes are used, or by the Esmarch tube-cultivation method. Of the three, the second method, in which Petri-dishes are employed, is the easiest and simplest to manipulate, since no levelling apparatus nor ice is required; but whichever method is employed, we commence in the same way. A tube containing approximately 10 c.c. of sterilised gelatin meat-broth is warmed just sufficiently to render its contents liquid. The sample of water

to be examined is shaken up well, so as to evenly distribute the organisms it contains, and, if the sample be a fairly pure one, 1 c.c. of it is measured out by means of a sterilised pipette, and added to the gelatin medium. The plug is then inserted in the tube, and the water intimately mixed with the bouillon by turning and twisting the tube about. A sterilised glass plate is then coated with one-half of the contents of the tube in the manner described on p. 238; when this has solidified, a second plate is similarly coated with the remaining half of the bouillon. If, instead of using cultivation-plates, we employ Petri-dishes, two sets of these are sterilised in the hot-air oven and allowed to cool. Into the lowermost dish of one of these sets half the contents of the infected tube are poured, and the remaining half into the lower dish of the second set. As the top dish is only removed for the few seconds it takes to pour out the contents of the tube, the chance of organisms falling in from the air is practically excluded. The gelatin is then distributed in a thin even layer on the bottom of the dish by gentle rotation. Esmarch's tubes may be coated in the manner previously described on p. 241; they are not so suitable for this purpose as plates or dishes, for, if many liquefying bacteria are present, the colonies are very apt to run together.

In case the sample of water is somewhat impure, a smaller quantity than 1 c.c. must be taken for infecting the gelatin bouillon, say 0.5 c.c. When very impure samples have to be examined, it becomes necessary to employ a considerably less quantity than half a cubic centimetre. As smaller quantities than this cannot be conveniently measured, in these cases it is better to begin by diluting the water. For this purpose we take a small stoppered bottle which has been sterilised, place in it 4 or 9 c.c. of sterilised water, and then add 1 c.c. of the sample of water. The mixture is thoroughly incorporated by shaking, and 1 c.c. of it taken for infecting the gelatin tube. Obviously in the first instance we use 0.2 c.c., and in the second 0.1 c.c. of the sample of water for the infection, and these quantities must be taken into account in the subsequent calculation to 1 c.c.

Standard for Comparison.—The number of colonies which 1 c.c. of water yields is taken as a convenient standard for the purpose of comparison. Thus, if, on examining a certain sample of water, of which 1 c.c. had been taken to infect the gelatin tube, and if, of the two plates which had been run with it, the one yielded 218 colonies, the other 224, then such water would be said to contain 442 organisms per cubic centimetre. Obviously, if the sample had been previously diluted to such an extent that only 0.1 c.c. had been taken, then the numbers found on the two plates would require to be multiplied by 10, in which case the number of organisms contained in such a water would be 4420 per cubic centimetre.

Counting the Colonies.—For this purpose the apparatus invented by Wolfhügel (Fig. 29) is generally employed. It consists of a wooden base, surmounted by a blackened ground-glass plate, on which the cultivation-plate is placed, and over this a glass plate marked into squares with a diamond, so supported at the corners that it does not come in contact with the gelatin film

on the cultivation-plate. The sides of the squares engraved on the counting-plate are generally a centimetre (about three-eighths of an inch) long, and several of these squares are still further subdivided. The counting is performed with the assistance of a magnifying-glass; the number of colonies in a certain number of squares are counted, and the average contained in each square estimated from these. The number so found, multiplied by the number of squares which are equal to the size of the gelatin film, gives the number of bacteria contained in the quantity of the water used for infecting the gelatin and which are capable of growing in this medium. The colonies formed in a

FIG. 29.—Wolfhügel's Counting Apparatus.

Petridish may be similarly counted by placing the dish on a sheet of black paper ruled in squares with Chinese white.

Biological Examination of Filtered Waters.—Where the water employed in a brewery is of such an impure nature as to necessitate its filtration prior to use, the biological examination of such a water, after filtration, affords valuable information, in the first instance, as to whether the filter is properly constructed, and capable of efficiently dealing with the quantity of water required; afterwards it is of great value in ascertaining if the filter is performing its functions properly. This is the one case where that cultivation method in which meat-broth gelatin is employed, and which is the one which reveals the total number of bacteria contained in a water, may be used with advantage in the practical working of a brewery.

Hansen's Method for the Bacteriological Examination of Brewing Waters.—It has been pointed out by this distinguished physiologist, to whom we owe so much for the elucidation of many points connected with the science of brewing, that the vast majority of the organisms found in water by Koch's method (meat-broth gelatin) have no significance whatever for the brewer. Very few of those which are capable of living and multiplying either in peptone meat-broth or on a gelatin meat-broth plate are capable of vegetating in such liquids as wort or beer. The conditions in these two last liquids are extremely unfavourable to the life of many bacterial organisms, since they are always slightly acid, and also contain some of the constituents of the hop, which are directly bactericidal in their action. Endeavours have been made to estimate the quantity of those bacteria contained in a water which are injurious to wort or beer, by the plate method of Koch, using, instead of meat-broth gelatin, ordinary boiled wort in which 10 per cent. of gelatin has been dissolved. It was found, however, that many organisms which were capable of developing

on a gelatin-wort plate would not grow in wort itself; hence this method, also, did not afford reliable information as to the number of bacteria which were capable of growing in wort. As Hansen suggests, the real question which the brewer has to decide is, "How does a water behave with reference to the wort and the beer; how many micro-organisms does it contain which are able to develop in those two liquids; and are there amongst them any species capable of causing disturbances in the brewing operations?"

The brewer obviously holds a much more advantageous position in the study of those bacteria which concern him than the hygienist does. The latter would be able to obtain much more precise and satisfactory information concerning the action of the individual organisms which he isolates on the human economy, if he were able to try their effect on the human body itself; but since he is obviously debarred from such a proceeding, he has to content himself with inventing methods for their identification, and trust to chance for the occurrence of cases where he can study their action on the human body. The brewer, in investigating the action of micro-organisms on wort and beer, is able to experiment directly with these very liquids themselves, and carry out his experiments under conditions which resemble as closely as possible those which actually exist in the usual routine of the working of a brewery.

In the actual examination of a water by Dr. Hansen's method, fifty flasks, each having a capacity of about 20 c.c., are taken and divided into two groups, A. and B. Into each of the 25 flasks of group A. 10 c.c. of wort are introduced; and into the remaining 25, which constitute the B. group, the same quantity of beer. All the flasks are plugged with cotton-wool and sterilised. When the flasks and their contents have become cold, one measured drop (0.04 c.c.) of the water under examination is added to each of fifteen of the flasks belonging to group A., and also to fifteen of the flasks of group B. To each of the remaining ten flasks of each group 0.25 c.c. of the water is added. The whole of the flasks are then shaken, so as to distribute the organisms, and kept at a temperature of 24° to 25° C. (75° to 77° F.) for fourteen days. At the expiration of this period the flasks are examined for signs of turbidity, and those which exhibit this sign of infection counted. Taking into account the total quantity of water added to the ten flasks, the number of flasks infected, and the total number of flasks taken, we find the number of organisms presumably present in this quantity of water, and from this is calculated the number present in each cubic centimetre of the water. Thus, if five out of the ten wort flasks (group A.) to which 0.25 c.c. of the water had been added have become turbid, then, as the total amount of water added to these ten flasks was $0.25 \times 10 = 2.5$ c.c., this quantity presumably contains five organisms¹ capable of developing in wort; consequently, the

¹ This is under the supposition that only one germ or organism is introduced into each flask with the 0.25 c.c. of water, which, in the vast majority of cases, is undoubtedly true, when a large number of the flasks remain unaffected. There is always, however, the *possibility* that two or more germs or organisms may have been introduced into one flask.

water contains two such organisms per cubic centimetre. If, as sometimes happens, the whole of these ten flasks have become turbid, no conclusion can be formed from them. We then turn to the remaining fifteen flasks of each group, to each of which only 0.04 c.c. of water had been added, and apply the same method of calculation. Thus, supposing that twelve out of the fifteen show signs of infection, then $0.04 \times 15 = 0.6$ c.c. of the water presumably contains twelve germs; consequently, one cubic centimetre of such a water will contain twenty organisms capable of developing in wort. The same process of calculation is applied to the flasks of group B., and the number of organisms per cubic centimetre of the water capable of developing in beer similarly found.

A comparative experiment performed by Dr. Hansen with the water supplied to the Old Carlsberg Brewery will serve to illustrate these differences. A plate culture was made after the method of Koch (gelatin meat-broth), using $\frac{1}{2}$ c.c. of the water. At the end of fourteen days there were found on this 111 colonies, that is, 222 organisms per 1 c.c. water, all of which consisted of bacteria. Very few of the colonies had liquefied the gelatin. Another plate-cultivation was made simultaneously, using wort gelatin. It was found at the end of fourteen days that fifteen colonies had developed on it, and as $\frac{1}{2}$ c.c. of water was used, this would be at the rate of thirty colonies per c.c. At the same time one drop (0.04 c.c.) of a mixture of equal parts of the water and sterilised wort was added (i.e., 0.02 c.c. water) to each of a series of fifteen wort flasks and to an equal number of beer flasks. These were kept for fourteen days and then examined, when it was found that the contents of none of the flasks showed signs of infection. In a second similar experiment, where the water yielded by Koch's method 1000 colonies per c.c., and the wort-gelatin plate showed thirty-four colonies, but two of the wort flasks became infected. One of these had developed a growth of bacteria, the other a growth of *Penicillium glaucum* (one of the commonest mould fungi), that is, 6.6 organisms per c.c. of water. The whole of the beer flasks remained unaltered.

Holm gives the following results of three comparative experiments of a similar nature, all of which are reckoned upon 1 c.c. of water:

—	I.	II.	III.
Koch's method .	8000.0, mostly bacteria.	350.0 bacteria.	370.0, bacteria.
Wort-gelatin plate	14.0, mould fungi.	8.0, mould fungi.	4.0, bacteria.
Wort . . .	{ 5.4, bacteria and mould fungi.	5.8, bacteria and mould fungi.	1.1, mould fungi and <i>Torulæ</i> .
Beer . . .	0.8, mould fungi.	0.8, mould fungi.	0.4, mould fungi.

The last example is worthy of note, since it shows that those species which developed in the wort and beer entirely differed from those that developed either in gelatin meat-broth or wort-gelatin plate cultures.

The following results are taken from a large number of analyses of brewing

water which were made by Professor Schwackhöfer. They likewise serve to show the remarkable differences in the behaviour of the organisms contained in the same water under the various methods of culture. The results of the experiments with wort and beer are given in percentages of the number of flasks which became infected. As 4 c.c. of water was added, collectively, to each hundred flasks, the numbers contained in columns three and four, divided by four, will give the number of growths per c.c. water :

No. of Colonies per c.c. Water.		Per Cent. of Flasks Showing Growths.		Opinion Formed from the Biological Examination.
Koch's Method.	Wort-Gelatin.	Wort.	Beer.	
56	o	o	o	Good.
165	o	o	o	Good.
609	o	o	o	Good.
3260	o	o	o	Good.
4134	o	8	o	Good.
6120	o	8	o	Good.
6000	o	80	8	Unfavourable.
8456	o	20	19	Unfavourable.
46,700	o	70	o	Permissible.
121,687	o	100	60	Bad.
769,780	o	100	4	Bad.

Wichmann's Method.—By this method, which is a modification of Hansen's, it is sought to give a numerical expression for the degree of vital energy possessed by the organisms existing in a water. In examining a number of samples of water by Hansen's method, in some cases it is found that the whole of the flasks become turbid on the first day, in other cases only after the expiration of three days, and in others only after a longer period than this. In those cases where the turbidity appears most rapidly it is assumed that the organisms possess the most vital energy, and that they possess less amounts as the length of time increases before which they afford visible indications of their presence. Hansen's method affords no information as to the amount of vital energy the organisms possess, and this deficiency Wichmann endeavours to remedy.

He proceeds in the following manner:—Twenty-five small flasks, each of which contains 10 c.c. of sterilised wort, are taken (group A.), and a like number, each of which contains the same quantity of sterilised beer (group B.). To twenty flasks of each of these groups a measured drop (0.025 c.c.) of the water under examination is added, and to the remaining four flasks of each group respectively 1.0, 0.75, 0.5, and 0.25 c.c. of the water; the remaining flask of each group being kept as a check on the sterility of the liquids employed.

These latter four flasks are numbered respectively 1, 2, 3, 4, and the time is noted at which each becomes turbid. If, with a certain sample of water, all these four flasks become turbid within twenty-four hours, then such a water is considered to possess a degree of impurity expressed by 100. This

number is arrived at by multiplying the numbers of each flask 1, 2, 3, 4, which stand in a sort of inverse ratio to the varying quantities of water added, by 10, and this is the factor with which the number of any flask is to be multiplied by in which turbidity shows itself in one day. Thus, where all are affected on the first day, the calculation is $(1 \times 10) + (2 \times 10) + (3 \times 10) + (4 \times 10) = 100$. In those cases where turbidity appears in any flask in two days, the factor to be employed is 8; if in three days, 6; in four days, 4; and in five days, 2. The general method of calculation is shown in the appended example:

No. of Flask.	Appearance of Turbidity.	Factors.
1. Infected with 1.00 c.c. water	2 days	$1 \times 8 = 8$
2. Infected with 0.75 c.c. water	3 days	$2 \times 6 = 12$
3. Infected with 0.50 c.c. water	3 days	$3 \times 6 = 18$
4. Infected with 0.25 c.c. water	4 days	$4 \times 4 = 16$
The infective energy of the water towards wort is 54.		

The remaining twenty flasks, to each of which 0.025 c.c. of the water was added, are calculated by Hansen's method.

As beer is supposed for this purpose to have a resistance to the vitality of micro-organisms $1\frac{2}{3}$ greater than that of wort, in calculating the results with beer the number of the flask is multiplied by $1\frac{2}{3}$ (1.67) in addition to the factor for the length of time elapsed before turbidity makes its appearance. For example:

No. of Flask.	Appearance of Turbidity.	Factors.
1. Infected with 1.00 c.c. water	3 days	$1 \times 6 \times 1.67 = 10.0$
2. Infected with 0.75 c.c. water	4 days	$2 \times 4 \times 1.67 = 13.4$
3. Infected with 0.50 c.c. water	5 days	$3 \times 2 \times 1.67 = 9.9$
4. Infected with 0.25 c.c. water	5 days	$4 \times 2 \times 1.67 = 13.2$
The infective energy of the water towards beer is 47.2.		

Investigation of the Air for Living Organisms.—Many forms of complicated apparatus have been devised for this purpose from time to time, some of which are described in the chapter dealing with the number and variety of the organisms found in the atmosphere (p. 332). Now much simpler methods are employed, and these are, as a rule, sufficient for the purposes of the brewer. When the air of any particular locality, such as that of the cooler-room, the fermenting-rooms, or the store-cellars, is to be examined, a number of glass cylinders (or wide-mouthed bottles) are thoroughly cleaned, labelled, and the superficial area of the mouth of each ascertained and noted on its label. They are then closed with cotton-wool plugs, and sterilised either by steam heat or in the hot-air oven. Caps of sterilised paper are then tied over the plugs, and the cylinders or bottles taken to the locality where the observation is to be made. The caps and plugs are here removed, the plugs being placed temporarily inside the caps and the cylinders left standing open for an hour. The plugs and caps are then replaced, the particulars concerning each cylinder noted on its label, and the cylinders taken back to the laboratory, where the germs which have fallen into them may be brought to development

in several ways. Each cylinder may be filled one-fourth full of sterilised meat-broth gelatin, or of gelatin wort, either of which has been just warmed sufficiently to render it fluid. The cylinder is then held under a stream of cold water and turned and twisted about until the gelatin is spread over its interior in a thin even layer and becomes solidified; or the gelatin, after admixture with the germs, may be made into a plate-culture or poured into a Petri-dish. The Petri-dishes, after being sterilised, may be used to catch the germs in the first instance, sterilised gelatin being added afterwards. In whichever way the culture is made, it is placed in the incubating chamber and allowed to remain for two or three days, when the various organisms will have formed colonies. These are counted, and, by taking into consideration the area of the mouth of the particular cylinder or dish and the number of colonies formed, a simple calculation gives the amount of germs falling per square foot from the air in a particular locality. The individuals composing the colonies can be investigated by removing any particular colony with a platinum wire and examining the organisms by the usual bacteriological methods, or they may be cultivated by the hanging-drop method, or their action on such liquids as wort or beer studied.

The colonies of mould fungi are readily recognised by their branching mycelium, which spreads out from the borders of the colony in all directions, giving it a star-shaped appearance. Many of them throw up aerial spore-bearing hyphæ, which are distinctive for the various species. The yeasts form small compact colonies about the size of a pin's head, generally having well-defined borders; they are nearly always colourless, though occasionally colonies of the so-called red yeasts are met with. The bacterial colonies are smaller than those of the yeast fungi; they are not so compact, and present a glutinous appearance; some of them may be coloured (chromogenous bacteria), whilst others may have an iridescent appearance. Many species of bacteria secrete a proteolytic enzyme and as a consequence liquefy the gelatin medium.

CHAPTER IV

VEGETABLE BIOLOGY

THE brewer is, in the course of his operations, brought so constantly in contact with processes of a purely biological nature that it is highly necessary he should possess some knowledge of this branch of science. A short account of the elementary principles of biology is therefore given, together with a brief biological description of those organisms with which he is chiefly concerned.

The Living Cell.—This constitutes the foundation, or, as it may be termed, the unit of all living organisms. Among the lowest and simplest forms of vegetable organisms many are known which, though they consist of but one single cell, are able to carry on all the functions essential to plant life, such as respiration, nutrition, reproduction, &c. As we ascend in the scale of vegetable life, the larger becomes the number of cells which enter into the structure of the plant; and very soon, instead of all the cells being alike and performing similar functions, they become divided into groups, to each of which a particular set of functions is assigned. Thus a highly organised plant, such as the hop plant, may be regarded as a vast congregation of groups of different cells, each group constituting a different tissue in the plant, and each having its own special set of duties to perform in the collective economy.

In commencing the study of the problems of plant life, we first devote our attention to those organisms which consist of a single cell, for here the conditions of life are presented in their simplest form.

Protococcus pluvialis.¹—This simple unicellular plant (Fig. 30) is described first, because, though it consists merely of a single cell, it performs all those biological functions which are essentially characteristic of the chlorophyll-bearing plants.

This organism is commonly met with in considerable quantities in the mud which collects in roof gutters, water butts, &c. When submitted to microscopical examination, the protococci are seen to consist of small round or oval bodies, each of which is surrounded by a tough resistant membrane called the "cell-wall," and this, by treatment with appropriate reagents, can be shown to consist of cellulose. Each of these little bags of cellulose is filled with a semi-

¹ Another closely allied species, *Protococcus viridis*, often found as a green film on the bark of trees, will serve equally well for examination.

fluid, jelly-like substance called "protoplasm," which constitutes the essential portion of the cell; living cells are known which consist of a mass of protoplasm without a protecting envelope. This remarkable substance is found in all growing cells, where it forms the seat of those vital actions which distinguish living from lifeless organic matter. Through its agency chemical decompositions and re-compositions of the most profound and complicated nature are constantly being carried on, with the most perfect regularity and apparently with the greatest ease; protoplasm possesses the power of absorbing many compounds, the elements or elementary groups of which it unlocks and rearranges into fresh states of chemical combination. These masses

FIG. 30.—*Protococcus pluvialis*.

(a) Adult condition ; (b) Reproduction by division, first stage ;
(c, d) Second and third stages ; (e) Zoospore.

of protoplasm, though in themselves extremely minute, are contained in such countless myriads in the members of the vegetable kingdom distributed throughout the world, that, taken collectively, they constitute one of nature's greatest laboratories, in which transformations, the magnitude of which is immense, are continuously being carried on.

Protoplasm, when moderately magnified, is seen to be of a more or less granular nature,¹ according to the age of the cell, being less granular in young cells, more so in old. It is not homogeneous throughout the cell; the portion nearest the cell-wall is somewhat denser and more translucent than the portion occupying the interior of the cell. The composition of protoplasm is not known with exactitude; it is undoubtedly extremely complex, and is of a proteid nature. In the cells of the *Protococcus*, and in almost all other vegetable cells, a small round body, the nucleus, may be observed. This seems to be concerned, in some obscure way, in guiding and controlling the functions of the cell; for instance, the division of the cell is always preceded by the division of its nucleus. Chemically it differs in composition from the protoplasm by containing nuclein, a substance into the composition of which phosphorus largely enters. In the interior of most active cells, especially when fully grown, there are to be seen one or more round or oval spaces, the vacuoles. These are filled with the cell-sap, which fluid also permeates the protoplasm and the cell-wall. A number of green or red granules are diffused throughout the protoplasm of the *Protococcus*; these are the chlorophyll cells, which play such an important part in the nutrition of this and other green plants.

¹ Protoplasm is much more easily examined in the organism next described, the yeast plant.

Food.—When the substance of a chlorophyll-bearing plant is analysed, it is found to consist of the elements carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur, together with minute quantities of potassium, magnesium, calcium, and iron. This leads at once to the supposition that all these elements must be present in the food of such a plant, if it is to carry on its vital functions efficiently. It has been proved experimentally that this is so, for if a plant like the *Protococcus* be grown in a solution which lacks any one of these elements, it gradually sickens and dies. In addition to this, we shall find, as we proceed further, that it is also requisite that these elements should be presented to the plant in such forms of combination as the plant can assimilate, and plants differ considerably with regard to the compounds they are able to appropriate for the purposes of nutrition; like animals, they exhibit considerable choice in the nature of their diet.

Assimilation.—Plants which possess chlorophyll cells are able to obtain the carbon necessary for their nutrition from the carbon dioxide of the air. The process by which they accomplish this is effected through the agency of the chlorophyll cells, and is called “assimilation.” That this process goes on in the *Protococcus* may be demonstrated by the following experiment: A strong test-tube is three parts filled with mercury, the remaining fourth being filled with water containing sufficient *Protococci* to render the liquid distinctly green; the whole is then inverted and placed in a dish of mercury. Carbon dioxide gas is now passed into the tube in quantity sufficient to displace about a fourth of its contents. The whole arrangement is placed in *bright sunlight* for several hours, at the expiration of which it will be found that the greater portion of the carbon dioxide originally present in the tube has been replaced by an equal volume of oxygen. This is shown by passing a piece of caustic potash into the tube, which dissolves in the layer of water, and absorbs the small quantity of carbon dioxide which has not been decomposed by the plant. If a solution of pyrogallol be then passed into the tube, the remaining gas, which is nearly as large in volume as the gas introduced into the tube, will be absorbed; this proves that it is oxygen. The little plant evidently takes in carbon dioxide, deprives it of its carbon, which it holds back for its own use, and rejects the oxygen. It is known that, in order to decompose carbon dioxide into its constituent elements, a large amount of energy is required. This energy the plant derives from the luminous rays of the sun; hence the transformation only proceeds in the light. We know that the peculiar agents which bring about this change are the chlorophyll corpuscles, because all plants unprovided with them are incapable of effecting this transformation. The process is supposed to take place in the following manner by double decomposition:

A number of formaldehyde molecules may then suffer condensation, one of the hydrogen atoms of one of them being simultaneously transposed, and in

this way a carbohydrate formed. For instance, we may imagine the formation of glucose to take place in the following way :

That some reaction of this kind actually does take place is rendered extremely probable by the researches of Emil Fischer, who has actually produced a sugar by the condensation of formaldehyde; and Brown and Morris¹ have pretty conclusively shown that the first products of assimilation in plant life consists of one or more sugars. (See p. 49.) The protoplasm is able, in some unknown way, to transform these first-formed carbohydrates into other bodies having a much larger molecule, such as starch, cellulose, lignin, fat, &c.

Every pound of carbohydrate formed in this way represents so much stored-up energy, a portion of which is afterwards liberated and employed in performing the internal work of the plant, such as the building up of its tissues, effecting chemical transformations in the protoplasmic contents of its cells, &c.; and the amount of carbohydrate employed in this way has been estimated to be about a thirtieth part of the total amount formed by assimilation.

In passing it may be mentioned that an immense portion of the work performed in the world is obtained from the combustion of the carbon which has been stored up by plants through the agency of their chlorophyll cells. Many animals derive their energy from vegetable food alone; the carnivorous animals also obtain theirs in an indirect manner from the vegetable world, since they feed on their herbivorous fellow-creatures. Those immense stores of energy, our coal-fields, represent the energy of the sun's rays, which has been stored up through the agency of the chlorophyll cells of plants which lived countless ages ago.

Formation of Proteïds.—In addition to the formation of carbohydrates, the protoplasm of the *Protococcus* performs another important function, viz., the building up of the proteïds, or the very bodies of which it is itself composed. From what has gone before it will be remembered that these bodies contain, in addition to carbon, hydrogen, and oxygen, also nitrogen and sulphur; and the protoplasm of many plants is able to build up its proteïds from the carbohydrates and such compounds of nitrogen as nitrates or ammonia, which are of an entirely inorganic nature. The *Protococcus* flourishes in rain-water, which always contains minute quantities of ammonia and nitrates. Hardly anything is definitely known as to how this is accomplished, but it is supposed that the ammonia or nitrate is first reduced in a manner somewhat analogous to that in which carbon dioxide is, and that in this reduced form it unites with a portion of the carbohydrates to form amides such as asparagine, glutamine, &c.; the amide then combining with a further portion of a carbohydrate to form a proteïd.

¹ *Journ. Chem. Soc., Trans.*, 1893, 63, 604.

This power of being able to construct proteid matter from purely inorganic substances, though peculiar to vegetable cells, is not possessed by all cells. No animal cell is capable of effecting this transformation, hence all animals have either directly or indirectly to depend upon vegetable organisms for their supply of proteids as well as for that of their carbohydrates.

Respiration.—All living beings breathe, that is to say, they take in oxygen (causing the oxidation of certain compounds, notably the carbohydrates, stored up in their tissues) and give out carbon dioxide. They derive in this way the energy requisite for performing the various functions of their life from the combustion of carbon. The liberation of energy by the combustion of coal in the boiler of the steam-engine is an analogous case. In the building up of a plant, a certain amount of work has to be performed, and this is obtained by the combustion of the carbon of some of its own carbohydrates. That the *Protococcus* respire may be shown by a modification of the experiment just described. If the test-tube be filled three parts full with mercury, the remaining fourth part with water containing *Protococci*, the whole inverted in a dish of mercury, and kept for several hours *in the dark*, a certain amount of gas will be evolved. If a piece of caustic potash be passed up the tube, the whole of the gas will be absorbed in a short time, showing that it is carbon dioxide. Since the gas is obtained from the combustion of the carbon of a portion of the carbohydrates contained in the plants by the oxygen dissolved in the water, its quantity may be taken as a measure of the energy expended in carrying on the collective life of the plants during the period of the experiment.

Excretion.—In addition to the constructive processes, a set of destructive processes are continually going on in the living cell. In these the protoplasm is broken down into simpler bodies, which, being of no further use to the plant, are thrown off or excreted. It is supposed that the oxygen taken in by respiration combines with the protoplasm, renders it unstable, and finally leads to its resolution into simpler substances.

Mineral Food.—Every plant when burnt leaves behind a certain quantity of mineral matter, technically known as “the ash.” This consists chiefly of the elements potassium, magnesium, calcium, iron, sulphur, and phosphorus, and it has been determined experimentally that all these substances are indispensable for the proper nutrition of all green plants. These elements, in the case of the *Protococcus*, are in the most suitable state for its nutrition when combined as the nitrates, sulphates, and phosphates of potassium, magnesium, calcium, and iron. The last element is only necessary to those plants which contain chlorophyll, iron being a constituent of that substance.

Osmosis.—Since all such plants as the *Protococcus*, which consist of a single cell, are closed in on every side by a cell-wall unprovided with any visible opening, it seems at first sight difficult to understand how such organisms obtain their supply of food from without. This they are enabled to do by the peculiar property which their cell-walls, and which mem-

branes generally possess, of permitting various substances in solution to pass or diffuse through them by a process called "osmosis." If a dry bladder is filled with water, the previously hard and rigid membrane becomes soft and pliable owing to the penetration of water into its substance; but the water does not pass through the bladder, it simply wets it. This peculiar property, exhibited by animal and vegetable membranes, of allowing liquids to permeate, without actually passing through them, enables such membranes to set up osmosis.

This process may be demonstrated in the following manner: A wide glass tube or cylinder, open at both ends (Fig. 30, *a*), is taken, and its lower end closed with a piece of bladder or parchment-paper. It is then partially filled with a strong solution of common salt or sugar, and supported in the vessel of water (*b*) at such a height that the level of the solution within the tube and that of the surface of the water outside (*c*) coincide. The level of the water in the exterior vessel should not, properly speaking, be above the edge of the parchment-paper, as it is difficult to make a perfectly gas-tight joint. The whole arrangement is left at rest for a few hours, at the expiration of which the level (*d*) of the liquid in the tube, as shown in the figure, will have risen considerably. The reason for this is that the water outside the tube permeates the membrane,

FIG. 30*a*.—Osmotic Apparatus.

and as the molecules of the salt present in the solution within the tube have a great attraction for the water molecules in the membrane, they draw them from the membrane into the tube. This passage of a liquid inwards is called "endosmosis." The membrane is also permeable to the salt solution, but to a much less extent than to water; consequently, the membrane is also wetted to a certain degree with the salt solution. The salt molecules present in this are attracted and drawn from the membrane by the water outside. This, which is the reverse of endosmosis, is called "exosmosis." What the relative proportions of these two actions shall be entirely depends upon the extent to which the membrane is permeable to the solutions with which it is in contact. Thus, in the preceding case, endosmosis is far greater than exosmosis, because the membrane is much more permeable to water than to salt solution.¹

If we conduct a similar experiment, closing the tube with a thin membrane of indiarubber, and using alcohol instead of salt solution, we shall find that the alcohol will pass through the membrane into the water, but that no

¹ The solution of substances in liquids is also due to the mutual attraction between their respective molecules. When a lump of sugar is placed in a vessel of water, and the whole allowed to remain at rest for some time, a perfectly homogeneous solution results; the sugar molecules rise up and become evenly distributed through the water molecules by virtue of their mutual attraction, in spite of the fact that the sugar molecules are held down by the force of gravity.

water will pass through the membrane to the alcohol; indiarubber is permeable to alcohol, but absolutely impermeable to water. In the former experiment, as the salt solution rises in the tube, it is evident that a certain amount of pressure is developed, and in some cases this may be very great. This pressure can be more clearly shown by completely filling the tube with salt solution and closing its upper extremity with another piece of bladder. After the tube has stood in water for some time, both membranes will bulge out considerably, and if the upper one is pricked with a pin, a small fountain of salt solution will gush out, showing that considerable pressure has been developed inside the tube. It is in this way that the cells of living plants are kept in that state of turgidity which is essential for the efficient performance of their vital functions.

Crystalloids and Colloids.—Those bodies which are able to pass through a membrane by osmosis are termed “crystalloids”; amongst these the crystalline bodies diffuse very rapidly; others, such as peptone, with extreme slowness. Many substances when in solution are unable to diffuse through a membrane; for instance, if a solution of albumin (white of egg) is placed in the tube, and the tube placed in the water as before, none of the albumin will pass through the membrane into the outside water. All those substances, such as gelatin, gum, &c., which behave in a similar manner, are called “colloids” (glue-like bodies). It was Graham who first distinguished between crystalloids and colloids in 1861, but osmosis had been utilised on a practical scale by Dubrunfaut to eliminate the proteïds in the refining of beetroot-sugar. Some colloidal solutions are clear, but the majority are turbid and disperse light rays as if containing solid matter in suspension, though they leave no residue when passed through a filter. It has however been demonstrated recently by Siedentopf and Zsigmondy by means of an instrument known as an “ultra-microscope,” that solid particles are present in these colloidal solutions, which must therefore be regarded as merely a suspension of minute particles of solids in a liquid medium. The difference in the nature of bodies in this respect is one of great importance in plant life, for crystalloid bodies taken into the living cell are often transformed in its interior into colloids, in which state they are incapable of passing out of the cell until they are again changed into crystalloids. It is by endosmosis that the *Protococcus* is able to obtain its nutriment from the outside; since its cell-sap is richer in substances in solution than the water in which the plant is immersed, the molecules of the substances dissolved in the cell attract and draw in the molecules of water which permeates the cell-wall, along with the carbon dioxide and the salts dissolved in this water. On the other hand, the substances that are to be excreted are dissolved in the cell-sap which permeates the cell-wall, from which they are attracted and withdrawn by the water outside; but as the balance between endosmosis and exosmosis is in favour of the former, the cell is kept in a state of turgidity. The transference of carbon dioxide from without to within, and the converse with regard to oxygen, is explicable on the same principle.

It is, however, extremely questionable if the processes by means of which solid, liquid, and gaseous bodies are transferred from the outside of the living cell, and *vice versa*, can be completely explained on purely physical principles ; most probably some of those unknown manifestations of force which at present we designate vital are also at work. In experiments we can only employ dead membranes ; the cell-walls of growing plants are living tissues.

Reproduction.—In the resting stage, which may be looked upon as the adult condition of the *Protococcus*, reproduction takes place by cell-division (see Fig. 30, p. 250). First the nucleus divides into two, then a new cell-wall commences to form, which eventually divides the organism into halves (*b*). The nuclei of each half again divide, another cell partition being formed at right angles to the first (*c*), and the original cell is thus divided into four (*d*) cells, which then separate and become rounded off until they resemble the parent cell (*a*). It is in this way that cell-division usually takes place in the vegetable kingdom.

The *Protococcus* can also reproduce itself in an entirely different manner. When this is about to take place, the protoplasm contracts and shrinks from the cell-wall, and then commences to divide first into two, then into four, and sometimes into eight portions, each of which finally assumes a rounded form. These “zoospores,”¹ as they are termed, are then liberated by the rupture of the wall of the parent cell. Each zoospore becomes pointed (*e*), and throws out from the point fine hair-like projections called “cilia,” which, by their rapid lashing movements, propel the organism along. Sooner or later each zoospore draws in its cilia, secretes a cellulose covering, and comes to a state of rest, when reproduction takes place in the ordinary manner by cell-division.

FIG. 31.—Budding Yeast.

Dormant Vitality.—If the *Protococcus* is carefully and slowly dried, it preserves its vitality for a considerable length of time ; and in this condition it is very readily disseminated by currents of air, hence its almost universal presence.

The Yeast Plant.—When a trace of yeast is mixed with a drop of water and examined under the microscope, it is seen to consist of a number of round or oval bodies, either isolated or joined together in groups, and varying in length from 3 to 10 micromillimetres (Fig. 31). Each of these separate bodies is an individual plant, which consists of a single cell, surrounded by a thin transparent cell-wall of cellulose or some closely allied substance.

The interior of the cell is almost completely filled with protoplasm, which is almost transparent in very young cells, but becomes more or less granular

¹ These afford instances of naked cells ; they consist of a mass of protoplasm without any external covering whatever.

as the cells grow older. The protoplasm is soaked through with the cell-juice, which also permeates the cell-wall; it collects in one or more cavities in the protoplasm, forming spaces having the appearance of small bubbles; these are termed "vacuoles." Unlike the *Protococcus*, the yeast plant contains no chlorophyll; consequently its protoplasm is much more easily examined. By carefully crushing yeast diluted with water between a cover-glass and slip with the blade of a knife, some of the cells will be ruptured and their protoplasmic contents squeezed out. By applying a little pressure to the cover-glass with the point of a needle when the preparation is under the microscope, it will be seen that the escaped protoplasm is of an elastic jelly-like nature. It may also be noted that the cell-wall, after the rupture of a cell, contracts, and this shows that, when the cells are entire, a certain amount of internal pressure is exerted on the cell-wall by the contents of the cell. If a little staining reagent, such as indigo or methylene blue solution, is run under the cover-glass, the dead protoplasm of the ruptured cells will take the stain freely, whilst that of the majority of the unruptured cells remains uncoloured. This rejection of staining matter is peculiar to living protoplasm, for this substance when dead exhibits a powerful attraction for many dyes. The number of dead cells in a sample of yeast may be readily detected by this curious difference in the behaviour of living and dead protoplasm. It is only necessary to place a little of the yeast mixed with a drop of a dilute staining liquid, such as methylene blue, under the microscope, when the dead cells are readily detected by the deep blue colour they assume. The yeast cell also contains a nucleus, but this is by no means such a prominent object as it is in most vegetable cells. It can, however, be rendered visible by staining with osmic acid or hæmatoxylin and picric acid; and Hansen has distinctly seen it without any special treatment, such as staining, in yeast cells which have been growing for some time on the surface of wort. According to the observations of Jansens, the nucleus of the yeast cell divides previous to the division of the protoplasm of the cell taking place, either in the formation of a new bud or of spores.

Nutrition.—If the yeast plant is placed in a solution similar to that in which the *Protococcus* flourishes, but to which a little ammonium tartrate has been added instead of nitrate, though it will live for a time, it eventually perishes from starvation. Yeast possesses no chlorophyll cells, and therefore cannot obtain the carbon necessary for its nutrition from the carbon dioxide dissolved in the liquid. Here, then, is a most important distinction between plants which bear chlorophyll cells and the Fungi, to which yeast belongs, which do not. Carbon must be supplied to them in the form of a substance, such as a carbohydrate, which they are able to assimilate. If to the former solution a little sugar be added, the yeast will then grow and multiply freely, showing that it is now provided with all the elements necessary for its nutrition. The Fungi, being devoid of chlorophyll cells, are dependent upon those plants which bear chlorophyll cells for their supply of carbonaceous nutriment, and in this respect they resemble animals. In ordinary brewing

operations, the yeast obtains its carbonaceous food-supply from the sugar in the worts, and this is derived from the starch which has been manufactured by the chlorophyll cells of barley or some other green plant.

As the protoplasmic contents of the yeast cell contain nitrogen, this element must also be supplied to the plant in a form which it can assimilate, and yeast manifests certain peculiarities in this respect. The *Protococcus*, and the chlorophyll-bearing plants generally, are able to derive their supply of nitrogen from a nitrogenous compound of a purely inorganic nature such as potassium nitrate, but the yeast plant is quite unable to do this. If, however, ammonium nitrate be substituted for this salt, then the yeast increases and multiplies, showing that its wants in this direction are satisfied. If, instead of the nitrate, a compound of ammonium with an organic acid such as tartaric acid is employed, then the yeast grows much more luxuriantly; and a still further improvement in this direction is exhibited when an amide body, such as asparagine, is substituted for the ammonium tartrate. It has also been found that peptones, one of the series of products of the hydrolysis of the proteids, are readily assimilated and utilised by yeast; and here again the plant resembles an animal organism. Yeast, when growing in brewer's wort, obtains its nitrogenous food-supply chiefly from the amides present in the wort, and other degradation products of the proteids of the malt. It contains phosphorus in considerable amount, which it derives from the phosphates always present in wort. In addition to the foregoing substances, small quantities of the elements magnesium, potassium, and calcium are indispensable, and wort generally contains these bodies in quantities over and above the requirements of the yeast.

Respiration.—The yeast plant, in carrying on its vital functions, expends a certain amount of energy, which it obtains by the oxidation of carbon through the instrumentality of the protoplasm. For this purpose a supply of oxygen, which the plant obtains by respiration, is necessary; it takes in oxygen and gives out carbon dioxide; but with reference to this process it exhibits a certain marked peculiarity. Since yeast has to spend a considerable portion of its existence in fermenting wort where it is entirely precluded from obtaining a supply of free oxygen, it is endowed with the power of taking in a store of this gas in order to bridge over those intervals. During these it derives the energy necessary for fulfilling its vital functions from the carbon burnt up by this stored-up oxygen. When an opportunity arrives, it again absorbs a fresh supply of the gas. In this respect, only in a greater degree, it resembles some of the aquatic air-breathing animals, such as the whale, which are able to take in a sufficient supply of air to enable them to remain under water for a considerable time. When yeast is grown under the conditions of complete exclusion of air, its growth gradually becomes more and more languid, and it finally dies.

Excretion.—The products of the breaking down of the protoplasm of the yeast, being of no further use to the plant, are excreted, and remain dissolved in the liquid in which the yeast has been growing. These, so far as has been

ascertained, consists of leucine, tyrosine, xanthine, guanine, and sarcine, bodies which have all been derived artificially from the proteïds by hydrolysis. The several enzymes which yeast secretes, and which have the properties, individually, of hydrolysing maltose to glucose, cane-sugar to invert sugar, &c., are also probably decomposition products of the protoplasm.

Fermentation.—Yeast possesses the remarkable property of exciting alcoholic fermentation, that is, of converting sugar into alcohol, carbon dioxide, and several other products. This faculty, which renders yeast indispensable to the brewer, is only incidentally referred to here, but it is one which will be dealt with at length subsequently.

Reproduction.—Yeast has two distinct methods of reproduction; the first of these is by budding; hence the name "*Budding Fungi*," which is often applied to the organisms of this class. The second is by the production of spores, which form in the interior of the cell.

When yeast grows in a liquid containing an abundance of food, it invariably increases by budding. As more food is absorbed by the yeast cell than is again destroyed in yielding the energy required for carrying on its vital functions, there is a gradual accumulation of substance in the interior of the cell. Its contents, therefore, gradually increase in bulk, and as the cell cannot grow beyond a certain size, some provision must necessarily be made for this surplus. This is effected by the protrusion of a bud, which gradually enlarges, and finally separates from the parent cell. When budding is about to take place in a cell, a small portion of the cell-wall becomes softened at some particular point, probably by the action of an enzyme secreted by the protoplasm. The protoplasm then pushes the softened portion of the cell-wall before it, and forms a small prominence (Fig. 31), which gradually increases in size to form a bud, *c*. This bud, when it has nearly attained the size of the original cell, becomes detached, *f*, and forms a separate individual, which, in its turn, reproduces itself by budding. In some species of yeast the newly developed plant does not separate from the parent cell immediately it reaches its maximum size, hence a number of cells remain attached to one another for longer or shorter periods, forming cluster-like groups.

Sporulation of Yeast.—When yeast cells are placed under certain conditions they cease to increase by budding; a series of changes then commences in the protoplasmic contents of some of the cells, which ends in the formation of spores within them. This phenomenon was observed by Schwann as early as 1837, and afterwards described in detail by de Seynes. Rees in 1870 found that several species of yeast were able to form endogenous spores. His method of procedure was to spread yeast, which had been washed with water, in a thin layer on slices of carrot, potato, &c., which were then covered with a bell glass. The preparations were in this way protected from dust and exposed to a moist atmosphere. He found that the cells increased by budding for a short time, then some of them died, whilst the younger and more vigorous ones, which were now filled with very

granular (foam-like) protoplasm and were well vacuolated, ceased to bud. The vacuoles disappeared gradually but completely, and in two or more places a condensation of the protoplasm took place, forming round islands. These now became surrounded with a membrane, that shortly afterwards thickened, and completely isolated the daughter cells (spores) from the remaining protoplasmic contents of the mother cell. This last was found to have increased considerably in size during the process (from $8-9\mu$ to $11-14\mu$ in diameter), the spores being from about 4μ in diameter. As this method of spore formation was similar to that observed in the Ascomycetes, he included the yeasts among these, giving them a distinct name, the Saccharomycetes.

This property of forming spores was afterwards found to be of great service in the differentiation of the various species of yeasts. Hansen pointed out¹ that the necessary conditions for obtaining abundant spore-formation were: the use of young and vigorous cells; that these should be kept on a moist surface; that they should have a plentiful supply of air; and that they should be maintained at a suitable temperature, 25°C . being the most generally useful one. The first of these conditions may be secured by cultivating the yeast for some time in beer wort, then introducing a few drops of this culture into a fresh stock of wort, which is kept at a temperature of 25° to 26°C . (77° to 79°F .) for twenty-four hours. A small portion of the yeast so grown is then spread in a thin layer on a block of plaster of Paris, cast in the form of a truncated cone, the lower portion of which is immersed in distilled water, and the whole kept at the required temperature. Blocks of baked clay, or portions of filter paper, have been employed by some investigators in the place of gypsum blocks; but these do not appear to offer any advantages. Sooner or later, according to the nature of the yeasts,

FIG. 32.--Yeast Spores (Hansen).

the temperature, &c., a series of changes begins in the protoplasmic contents of the cell, which ends in the formation of from one to six or eight glistening cells, which are called spores (Fig. 32). These when placed in a suitable nutritive medium begin to germinate by throwing out one or more buds, which eventually become yeast cells.

The newly-formed yeast cells then reproduce themselves in the ordinary manner by budding (Fig. 33). Klebs put forward the view that one of the most important conditions for spore-formation was that the yeast must be entirely deprived of food. Barker² investigated the conditions of spore-formation very closely, and found that with some yeasts, of which *Sacc. cerevisiæ* I. (Hansen) is an example; this statement of Klebs was to a great extent true, for most spores were formed when the yeast cells were entirely deprived of nourishment. With other yeasts, such, for instance, as *Sacc. anomalus*, the case was entirely reversed;

¹ *Med. Carlsb. Lab*, 2, 2, 1883.

² *Journ. Fed. Inst. Brewing*, 1902, 8, 26

when totally deprived of nourishment this yeast failed to yield spores, but produced them in abundance when liberally supplied with nutriment. Barker also showed that the amount of moisture supplied to the cells under culture was without influence, provided the cells were not completely desiccated. He also found that the presence of carbon dioxide had a most prejudicial influence on spore-formation; hence one of the principal functions of abundant aëration was the speedy removal of this gas. He also confirmed

FIG. 33.—Germination of Yeast Spores (Hansen).

Each letter in the figure refers to the same cluster of spores in the different stages of development.

Hansen's statement, that a necessary condition for abundant spore-formation is the employment of young and vigorous cells.

Spore-formation in the *Saccharomycetes* may be a sexual act. Schöning¹ observed that the asci of *Schizosaccharomyces octosporus* (see p. 264) were formed in a peculiar manner; a septum first formed in the cell, then around this a constriction formed which proceeded until the two daughter cells were united by a small neck. The remains of the septum in the neck then disappeared, and the two cells, instead of separating, fused together to form a large ovoid cell in which the first signs of sporulation made their appearance. Hoffmeister showed that here a sexual process took place. By staining, he found that each daughter cell contained a nucleus, and that these nuclei, after the fusion of the two daughter cells, approached each other and finally coalesced. This large nucleus afterwards split up into eight segments, each of which condensed protoplasm around it to form spores. Barker² described the process of sporulation in a yeast he had found on

¹ *Med. Carlsb. Lab.* 4, 1.

² *Proceedings Royal Society*, 68, 346.

ginger, and which he named *Zygosaccharomyces*. This yeast increased under normal conditions by budding; it could ferment maltose, glucose, and sucrose. When a number of cells were observed in a hanging drop of water, the protoplasm soon began to assume a very granular appearance, and in twenty-four to forty-eight hours buds commenced to form, which gradually extended into tubular beaks. The beaks of two adjacent cells having grown towards one another until they touched, union took place. The cell wall at the conjugating tips disappeared, and a tubular connection from one cell to the other was established. The usual changes then took place in the protoplasm resulting in the formation of (as a rule) two spores in each cell. Guilliermond, who also studied spore formation in hanging drop cultures, was able to confirm Schöning's observations on *Sacc. octosporus*, but found that this process was not the usual one. Generally it took place in a manner similar to that of the *Zygosaccharomycetes*. A similar process was also observed in the *Schizosaccharomyces pombe*; but instead of one large cell being formed by the coalescence of the two daughter cells, these remained attached by a narrow neck. In these cases there is, therefore, that actual fusion of the nuclei which is always regarded as a sexual act. The principal rôle in spore formation is sustained by this fused nucleus, and probably each of the spores formed contain a portion of it. The process may, therefore, be regarded as a sexual one, being a very simple form of isogamy, such as that which is seen in the Mucors (page 277).

Some time before the above observations had been made, Jannsen and Leblanc¹ described what they considered to be a sexual process in *Sacc. cerevisie* and some other yeasts. They found that in the earliest stages of spore-cultivation the nucleus divided into two portions, which moved a short distance away from one another, then approached and finally coalesced. The nucleus so formed now divided into segments, each of which became the nucleus of a spore. Barker² made spore-cultivations of *Sacc. cerevisie* I. on gypsum blocks at a temperature of 25° C. From the first to the twenty-fourth hours specimens were removed and stained preparations made from them, and the appearances observed serve to a great extent to confirm the views of Jannsen and Leblanc.

Species and Varieties of Yeast.—For a long period the yeasts were arranged in various species according to the differences in form which they exhibited when viewed under the microscope. Those of a more or less spherical shape were termed *Saccharomyces cerevisie*; those of an elliptical form, *Saccharomyces ellipsoideus*; those having a sausage-shaped form, *Saccharomyces Pastorianus*. After Hansen had introduced his method of growing yeast from a single cell, by means of which it first became possible to secure cultivations which consisted absolutely of one variety, it was found that any method of distinguishing the species of a yeast from its shape alone was misleading, for each species of yeast was able, under different conditions of cultivation, to assume the forms of the others.

¹ *La Cellule*, 14.

² *Journ. Fed. Inst. Brew.* 1902, 8, 65.

Hence other and more perfect methods of discrimination had to be devised such as that which depends on the differences in the time taken by the various species of yeast to form spores at the same temperature, described farther on. It was also found that many organisms which did not form spores had, from their close resemblance in appearance to the yeasts, been included under the denomination *Saccharomyces*. It was now, however, determined to restrict this designation to those organisms only which were able to produce spores.

Saccharomyces cerevisiae.—Under this term are included those yeasts which have been employed from time immemorial in brewing operations, and which are, therefore, spoken of as the “cultivated yeasts.” They are divided into two great classes—the top fermentation and the bottom fermentation yeasts, so called because the former rise and collect on the surface of the fermenting fluid, whilst the latter fall to the bottom. Top and bottom yeasts were at one time regarded as distinct species for, after much experimenting, it had not been found possible to convert the one into the other. Of each of these two forms there are many varieties, which differ remarkably in their respective properties, such as the degree of attenuation they are able to carry the wort down to, the flavour of the beer they yield, &c. The top fermentation yeasts readily form spores, the bottom ones only with difficulty.

The Wild Yeasts.—In contradistinction to the cultivated species, there are numerous other yeasts met with in Nature, some of which occasionally intrude themselves into the brewery, and there cause trouble. Among these are Hansen’s *Saccharomyces ellipsoideus* I. and II., also *Saccharomyces Pastorianus* I., II., and III., and several others. Some wild yeasts exhibit a great tendency to grow on the surface of the liquid in the form of a film, hence it would appear that they require a free supply of air for their welfare. Many of them are able to ferment maltose, glucose, and cane-sugar; others cannot. One variety of the film-forming yeasts, *Saccharomyces anomalus*, readily causes fermentation in wort, to which it communicates a fruity odour. This yeast produces peculiarly-shaped spores, which have the form of a hat.

Saccharomyces apiculatus.—This yeast (Fig. 34) is readily distinguishable by its lemon-shaped cells; in fact, it is the only yeast which can be recognised with any certainty at all from its form alone. The buds invariably form at the pointed ends of the cells, and the newly detached cells preserve for some time the oval shape of an ordinary yeast cell. According to Grönlund, these yeasts which, when living in their natural state, pass a portion of their time embedded in the soil, during that period produce spores; they do not, however, yield spores when cultivated under artificial conditions. *Saccharomyces apiculatus* contains no inverting enzyme, consequently it cannot ferment any of the disaccharide sugars, but it readily ferments glucose and lævulose. This yeast occasionally invades the worts, especially in autumn, but it rarely produces trouble there unless it happens to be present in very large quantity.

The Schizo-saccharomycetes.—These yeasts do not throw off buds after the manner of the ordinary yeasts, but rather by a process of fission, such as that which obtains with the bacteria. The elongated cells, which somewhat resemble those of an *Oidium*, when about to divide, form a partition-wall at right angles to the long axis of the cell; the cell then breaks into two portions at this division-wall, and the square ends thus left become afterwards rounded (Fig. 35). To this genus belong the *Schizo-saccharomyces pombe*, isolated from

FIG. 34.—*Saccharomyces apiculatus* (Hansen).

FIG. 35.—*Schizo-saccharomyces pombe* (after Lindner).

millet-beer which is produced in Central Africa; and the *Schizo-saccharomyces octosporus*, so called from its tendency to form eight spores in each cell. These yeasts appear to be able to ferment some of the malto-dextrins of high molecular weight, which the cultivated yeasts are unable to do.

Mycoderms.—A very valuable contribution to this subject was made to the Institute of Brewing in 1901 by H. Van Laer.¹ The organisms which are classed under the head of *Mycoderma cerevisiae* are very similar in appearance to ordinary yeasts. They grow with enormous rapidity on the wort or beer when supplied with the necessary amount of air. Van Laer points out that if those characters which are employed as a means of classifying the yeasts are sufficiently constant to make the distinctions valid,

¹ *Journ. Fed. Inst. Brewing*, 1901, 7, 337.

the term *Mycoderma cerevisiæ*, like *Saccharomyces cerevisiæ*, can no longer be regarded as representing an individual species. In fact, among the *Blastomycetes* (budding fungi) which appear in the form of films on the surface of alcoholic liquids exposed to the air, there are some which can be reproduced by sporulation as well as by budding.

P. Lindner¹ describes these organisms under the name *Kahmhefen* (mycoderma yeasts) and divides them into two groups, sporogenous mycoderma yeasts, which may be termed *Saccharomyces mycoderma*, and which comprises the forms known under the names of *S. membranefaciens* (Hansen), *S. hyalosporus* (Lindner), *S. anomalus* (Hansen), &c.; and asporogenous mycoderma yeasts, or mycodermæ properly so-called. Almost all brewery yeasts contain some cells of this kind, and when any beer or yeast is taken at random, the species most likely to be obtained is that possessing the morphological and physiological character of *Mycoderma cerevisiæ* (Hansen).

When growing on the surface of an alcoholic fluid, the *Mycodermæ* decompose the alcohol into carbon dioxide and water. When immersed in a saccharine fluid, some of them, such as the *Mycoderma cerevisiæ*, are able to cause alcoholic fermentation, small amounts of alcohol (under 1 per cent.) being produced. As a rule, they appear to do no harm when growing on the surface of beer, though occasionally they cause turbidity.

The Torulæ.—These organisms were first described by Pasteur; they increase by budding after the manner of the *Saccharomycetes*, which they much resemble in shape; their cells are, as a rule, nearly spherical. The *Torulæ* are generally very much smaller than yeast cells, though occasionally they attain the same size. None of those described by Pasteur were able to induce alcoholic fermentation; but of the seven varieties studied by Hansen some secrete invertase, and a few of these, though not all of them, are able to ferment cane-sugar. It has been asserted by Claussen (see p. 388) that a species of torula which he names *Brettanomyces* causes the secondary fermentation and conditioning of British beers.

THE BACTERIA

The bacteria, are a class of exceedingly minute micro-organisms met with in the greatest abundance in nature. When an organic infusion of almost any kind, vegetable or animal, is left exposed to the air for a short time, it becomes invaded by hosts of these organisms. They are objects of considerable import to the brewer, for, as we shall see further on, they are capable of causing an immense amount of damage in many ways.

Bacteria vary much in shape, and may be divided, according to their form, into three principal classes. To the first of these belong those micro-organisms which, in a perfect state of development, have the form of little spherules;

¹ *Mikroskopische Betriebskontrolle in den Gärungsgewerben*. Berlin, 1905. (Paul Parey).

they are called *Cocci* (Fig. 36, *a*). Those of the second class have the shape of cylindrical rods, and are distinguished as *Bacilli* (*e*). The third class have the form of a spiral, and are called *Spirilla* (*f*). The three classes may be likened in form, as De Bary suggests, to a billiard ball, a lead-pencil, and a corkscrew. The bacteria are, so far as is known at present, the smallest organisms in nature, and require for their examination the highest powers of

FIG. 36.—Bacteria (after Baumgarten).

- (*a*) Cocci; (*b*) Diplococci; (*c*) Tetrads; (*d*) Sarcinae; (*e*) Bacilli; (*f*) Spirilla;
 (*g*) Bacteria with spores; (*h*) Bacteria with flagella; (*i*) Zoogloeae;
 (*j*) Staphylococci; (*k*) Streptococci.

the microscope. Being much smaller than yeast cells, the two are readily distinguishable one from another.

Structure.—Bacterial organisms consist of a mass of protoplasm surrounded by a cell-wall of cellulose; the outside of this envelope is coated with a mucilaginous layer more or less swollen by the absorption of water. In consequence of this, the outline of these organisms, as seen under the microscope, never presents that sharply defined border that a yeast cell does. The protoplasmic contents of bacteria are, as a rule, colourless; but to this there are exceptions. Some bacteria contain starch (granulose), and these are coloured blue on the application of a solution of iodine. The protoplasm of some bacteria is studded with grains of sulphur, and ferric oxide is deposited in the cell-walls of others.

Reproduction by Fission.—Bacterial organisms, like yeast, have two separate modes of reproduction. The first, and by far the more common

mode, is by division, and this takes place in the following manner: When a bacterium has grown to a certain length, a constriction, situated at right angles to the long diameter of the organism, appears in its middle; this gradually deepens, and at last divides the microbe into two separate individuals. Even in the micrococci a slight lengthening takes place before the organism divides. Owing to this peculiar method of reproduction, by splitting, as it were, the bacteria are collectively known as the *Schizomycetes* or *Fission Fungi*. In the *Bacilli* and *Spirilla*, division always takes place in a direction at right angles to the long diameter of the organism. In some of the micrococci the splitting-up proceeds simultaneously in two directions, the plane of the second division being at right angles to that of the first; in this way one micrococcus is converted into a group of four, arranged in the form of a square and called a *Tetracoccus* or *Pediococcus* (Fig. 36, c). In a few other species cell-division takes place simultaneously in three planes, all of which are at right angles to one another, and, as a consequence of this, small packets, each of which consists of eight micrococci, are produced (d); these are known as the *Sarcinæ* or *packet* organisms. In many bacteria which divide in the most usual way, that is, in a direction at right angles to their long axis, the two cells after division do not separate at once, but remain attached to one another for longer or shorter periods. In this way chains of bacteria are formed, each of which consists of at first two, then four, then eight individuals, and so on. When large numbers hang together in this way the chain often has the appearance of a long thread or filament. When micrococci remain attached to one another after division, the chain has the appearance of a necklace; such forms are called *Streptococci* (k). Sometimes the tendency to adhere only extends to the pair first formed; in this way we get the forms called *Diplococci* (b). When the micrococci separate immediately after they have divided, and lie alongside one another haphazard, they present the appearance of a bunch of grapes; such varieties are entitled *Staphylococci* (j). In describing the bacilli we often speak of the organisms as long or short, thick or thin bodied. Bacteria, especially when grown on the surface of liquids, often become united so as to form a tough gelatinous mass. In assuming this state the exterior mucilaginous coatings of the organisms become considerably swollen and finally fuse together, the bacteria being retained in a perfectly regular manner throughout the mass. This is known as the zoogleeal state (i).

Reproduction by Spores.—In addition to the method of reproduction by cell-division, bacteria, under certain conditions, form spores (Fig. 36, g). One of the chief of these is the exhaustion of the nutritive materials of the medium in which the organisms have been growing; but other special conditions are also necessary, and these vary considerably with the different species. The power of forming spores is almost entirely confined to the bacilli; it is only occasionally met with in the spirillæ, and some of the sarcinæ are also said to possess it. When a bacillus begins to sporulate, a portion of its protoplasmic contents assumes a granular appearance, and in this a cell-wall gradually forms,

which encloses a portion of the protoplasm; this gradually becomes more and more homogeneous, and eventually forms the spore. The contents of the spores are of a highly refractive nature, and consequently present a brilliant, glistening appearance when seen under the microscope. The motile bacteria invariably become motionless when about to sporulate. In some bacilli the spore forms in the middle of the protoplasm, the remainder of which is afterwards absorbed; in consequence of this the organism becomes spindle-shaped. In other bacilli the spore forms at one extremity, and when this is the case the organism acquires the form of a drum-stick. When the spore has completely formed, the remainder of the organism disintegrates and disappears, leaving the spore isolated. It remains in this condition until chance places it in a suitable nutritive medium, where, after a shorter or longer period of rest, it germinates, reproduction being afterwards carried on in the ordinary manner by cell-division. The membranes which invest the spores of bacterial organisms are of an exceedingly tough and resistant nature, and this enables the spores to withstand the action of agents which would rapidly prove fatal to the organisms when in their fully-developed state. Tyndall found that the spores of the hay bacillus (*Bacillus subtilis*) were capable of germinating after they had been boiled for several hours in water. As the spores of the bacterial organisms are extremely minute, they are disseminated and carried to considerable distances by air-currents, hence their universal presence in the atmosphere.

Nutrition.—As the vast majority of bacteria possess no chlorophyll, they are unable to derive their supply of carbon from the carbon dioxide of the atmosphere. According to Nägeli, they can appropriate the carbon of almost all compounds which contain this element, providing these are soluble in water and are not actually poisonous. Some bacterial organisms met with in the soil can even obtain their supply of carbon from carbonates. The bacteria are able to derive their supply of nitrogen from an enormously large variety of substances. One of the most suitable of these is a solution of a proteid, or, in those cases where the organism does not secrete a proteolytic enzyme, a solution of the hydrolytic products of the proteids. Substances of this nature may be replaced by amides such as asparagine, leucine, &c., or by ammonia and its salts, and in most cases an inorganic salt of nitric acid may take the place of ammonia. Some varieties of bacteria found in the soil are not only able to derive their own supply of nitrogen from the air, but also to hand over a certain quantity of it to certain crops, such as peas, beans, clover, vetches, &c. These plants form nodules on their roots in which these nitrifying organisms congregate. Consequently a soil is richer in nitrogen after a crop of any of these leguminous plants has been grown on it than it was before.

Respiration.—Many of the bacteria cannot carry on their vital function without a supply of free oxygen, but with others a small amount of this gas immediately impairs their functions, and a larger quantity proves fatal to the organisms. The former are termed “aërobian,” the latter “anaërobian.” A

third class, called "facultative aërobians," seem to occupy a middle position, they are able to functionate either with or without a supply of oxygen.

Excretion.—It is certain that the substances formed by the breaking down of the protoplasm of the bacterial organisms are excreted. Little is known as to the nature of these products.

Secretion.—Many of the bacteria are able to secrete enzymes of various kinds, proteolytic, amylolytic, &c. A number also secrete pigments of various colours, such as yellow, green, blue, orange, &c. The *Bacillus prodigiosus*, for instance, when grown on a starchy medium (such as a slice of moist bread), secretes a blood-red pigment.

Fermentation and Putrefaction.—The most remarkable property possessed by bacterial organisms is the power of inducing a number of profound changes in the media in which they grow. By virtue of this they are able to decompose an amount of matter enormously in excess of that which they require for their own nutrition. This extraneous chemical work is called "fermentation" when it is unaccompanied by an offensive odour, and especially when the products yielded are of a useful nature, as in the fermentation caused by the *Bacterium aceti*, where alcohol is transformed into acetic acid. When, on the other hand, the change is accompanied by an offensive odour, the process is called "putrefaction," and, as a rule, this takes place when the substratum is of an albuminous nature. In putrefaction a number of very malodorous compounds are usually formed, and in the case of putrefying animal substances, as is occasionally seen in tinned meats and fish, in cheese &c., bodies called "ptomaines" are produced, which are of an extremely poisonous nature. But the nature of the chemical changes which bacteria are able to effect in the medium in which they flourish are nearly as numerous as the organisms themselves, and this is truly remarkable when we consider that the essentials of plant life, that is, the protoplasm and cell-wall, are apparently identical in every organism. Thus we have bacteria which transform sugar into lactic acid; others which transform lactic acid into butyric acid; others convert alcohol into acetic acid; some effect the transformation of the ammonia present in the soil into nitrites; whilst another set carry this transformation a stage further, and convert the nitrites into nitrates. When organic substances have been broken down to a certain stage by one class of micro-organisms, the products of these are seized upon and further broken down by micro-organisms of another class, and this proceeds until at last the original matter is entirely converted into ammonia, water, and carbon dioxide, substances which are again available for the food-supply of a new generation of plants. The micro-organisms are, in fact, nature's scavengers, for, as Sachs observes, "If there were no fungi, the entire surface of the earth would be covered with dense layers of the bodies of plants and animals which had accumulated for thousands of years." If such layers had been permitted to form, they would long since have locked up in themselves the elements necessary for the building up of new plants and animals, and life, ages since, would have become an impossibility. Were micro-organisms suddenly

annihilated, the return to the atmosphere and to the mineral kingdom of all that has ceased to live would be totally suspended. It is by means of this peculiar power of effecting the decomposition of masses of matter enormously in excess of their own nutritive requirements, with which micro-organisms are endowed, that they are able to perform this absolutely necessary and indispensable work in the economy of nature. If they only appropriated the quantity of matter necessary for their own nutrition, the result would simply be an immense accumulation of the dead bodies of these organisms.

Bacteria are omnipresent, and have the unfortunate habit of intruding into places in the brewery where their presence is highly undesirable. They are invariably present on the surface of barley and malt, the former of which, when in a moist condition, forms an excellent breeding-ground for bacterial and other organisms. They also invade the wort and the finished beer. As the changes which bacteria bring about are often of an extremely prejudicial nature, one of the brewer's principal cares is to keep these invaders away from his materials as far as possible, and to hold those organisms in subjection which unavoidably do gain access to them. All accumulations of moist organic materials, such as damp malt, spilled yeast, moist grains, &c., form excellent breeding-grounds for bacteria. Such masses of matter afterwards become dry and get more or less pulverised. In this state they are liable to be carried away by currents of air and disseminated in all parts of the brewery, carrying with them the dried organisms, which often become deposited in places where they may cause considerable mischief. Cleanliness is one of the essential precautions for the brewer to observe in keeping down these objectionable intruders. Like yeasts, bacteria preserve their vitality for considerable periods when slowly dried, and, on account of their exceedingly minute size, they are especially liable to become suspended in the air and wafted about by air-currents.

Locomotion.—Many bacterial organisms possess the power of locomotion, but this is almost exclusively confined to the bacilli and spirilla, only one motile micrococcus, the *Micrococcus agilis*, being known. The motion is brought about by one or more whip-like filaments called "flagella," which are generally attached to the ends, more rarely to the whole surface of the organism (Fig. 36, *h*). Under the ordinary conditions of microscopic observation the flagella cannot be seen. They can, however, be rendered visible by particular methods of staining. They propel the bacteria along by their rapid undulating movements, and in some of these organisms the rate of motion is so great as to cause considerable inconvenience to the observer when examining them in the living state.

THE PRINCIPAL BACTERIA MET WITH IN BREWING OPERATIONS.

Fortunately for the brewer, of the large number of bacteria known but few are able to live in hopped wort, and many of those which can are unable

to survive the fermentation with yeast which the wort is subjected to in the production of beer; a few of the latter are able to exist in beer if they are added to it after it has passed through the primary fermentation. The reason for this is that yeast, when in an active state of fermentation, possesses the peculiar power of protecting itself against the attacks of bacterial organisms, and so much is this the case, that at the end of the primary fermentation very few living organisms beyond the yeast itself are to be found in the beer. The case is, however, exactly the reverse when the yeast is in a resting condition, such as it is in when stored for subsequent use. Those organisms, the attacks of which the yeast was so well able to resist when in the active state, now obtain the mastery: they exercise their fermentative actions upon it and set it into a state of decomposition. Those bacteria which are able to survive the fermentation appear to have little or no action upon yeast when in the resting-stage. Of the bacteria met with in wort and beer, some are aërobic, others anaërobic. The latter are the more dangerous class, since the conditions of rigorous exclusion of air under which beer is kept are without influence on them.

Bacteria which can Exist in Wort, but cannot Survive the Primary Fermentation.—Many of these, to which Hansen gave the generic name of "wort bacteria," are only able to thrive in unhopped wort. The bactericidal action possessed by some of the constituents of the hop, notably the soft resins, completely arrests the growth and development of many micro-organisms.

Bacterium termo.—Under this designation a number of organisms (Fig. 37) very similar in appearance, which invariably appear in great abundance in any vegetable or animal infusion that is left exposed to the air for a short time, have been confused. They are all motile. A number of these have been examined and described by Lindner¹ under the following names:

Termobacterium lutescens has the form of short rods about 1μ in breadth, pointed at the ends and slightly contracted in the middle. Under certain culture conditions it develops involution forms, which consist of indented chains or of long filaments.

On meat-broth gelatin plates, the colonies have at first sharp, well-defined borders, but afterwards throw off radiating limbs. The gelatin is liquefied in five or six days, the culture being kept at the ordinary temperature. They grow rapidly in wort at a temperature of 15°C . (59°F .), and cause it to become turbid in twenty-four hours; a film then forms on the surface of the liquid at its margins, and a pronounced odour of celery is developed. In four weeks the pellicle has increased to a thick, yellow film, the wort is then clear, but a considerable glutinous deposit of a dirty brownish-yellow colour has formed. In beer which had been infected with a vigorous culture

FIG. 37.—*Bacterium termo*. The pair seen in the lower part of the figure are more highly magnified and stained, so as to show the flagella.

¹ *Mikroskopische Betriebskontrolle Gärungsgewerben*, 1905, p. 461.

of this bacterium, and which had been preserved in a well-corked bottle for two and a half months at a temperature of 50° F., some of the organisms were found alive, and this shows that, when added to beer after fermentation, they are able to survive. When, however, they were added to wort previous to fermentation, all were found to have perished at its termination. A few of the bacteria added to pressed yeast caused it to putrefy in a few days.

Termobacterium fuscescens.—This bacterium closely resembles *T. lutescens* in appearance, motility, and the power of causing turbidity in wort and in developing an odour of celery. It is specially characterised by its ability to form lactic acid in hopped wort and beer. Old cultures on wort-gelatin had a brownish colour, surrounded by a zone of similarly coloured gelatin; hence the name of the bacterium.

Termobacterium iridescens.—This bacterium, when cultivated in wort, causes turbidity and a celery-like odour; hydrogen is also evolved in small bubbles. Unlike its congeners, it is immotile. On meat-broth gelatin it forms colonies which have a bluish, iridescent colour, somewhat resembling fish-scales. It does not grow nearly so vigorously on wort-gelatin.

Termobacterium erythrinum.—Grows luxuriantly in wort, on which it quickly forms a reddish film. In shape it resembled the *T. iridescens*, the cells being generally grouped in pairs, more rarely in fours. It does not grow readily on meat-broth gelatin, but exceedingly well on wort-gelatin, which it liquefies in a short time. When growing in wort or glucose solution, a slight evolution of gas is observed.

The Butyric Acid Ferments.—These rod-shaped organisms (Fig. 38) were first studied by Prazmowski, who, under the supposition that they were of

FIG. 38.—Butyric acid Bacteria.

one species, termed them *Clostridium butyricum*. They readily form spores. From the more recent observations of Beyerinck¹ there are several species of butyric acid ferments, some of which he describes under the designations *Granulobacterium butyricum*, *G. saccharobutyricum*, *G. lacto-butyricum*, *G. polymyxa*. They are all facultative anaërobic organisms, and, when grown under exclusion of air, become either partially or completely filled with granulose; hence their name. When

in this condition they are coloured blue by iodine. An exceedingly unpleasant rancid odour is developed in liquids in which they are present. As they are very sensitive to the bactericidal action of the hops, and require a somewhat high temperature for their development (about 122° F.), they are but rarely met with in brewing operations.

Bacillus viscosus, No. 1 and No. 2.—Under these names Van Laer has described² two species of rod-shaped organisms which are able to induce what is termed "ropiness"³ in wort and beer. They resemble one another closely

¹ Koch's *Jahresbericht*, 1893, p. 258.

² *Zeit. ges. Braw.*, 1890, p. 11.

³ When beer enters into this condition, it at first becomes turbid, and afterwards so viscid that it resembles oil when poured from one vessel to another. Later on the viscosity

in appearance, and when grown on meat-broth gelatin, form brownish-coloured colonies with well-defined borders. They do not liquefy gelatin. No. 1, when introduced into sterilised beer wort, induces viscosity in twenty-four hours, and in forty-eight hours the wort is converted into a viscid mass as coherent as white of egg, carbon dioxide being meanwhile evolved. The surface of the wort becomes studded with pale-yellow, glutinous, branched patches. In cultivations of No. 2 this last appearance is absent, and there is less evolution of gas. In closed bottles the first variety forms mucilaginous masses, in which the rods sporulate; the second variety does not behave in this manner. The ropy condition is not induced in worts at temperatures below 44° F.; the action proceeds most rapidly at 91°, and is arrested at 107° F.

Bacteria which are Able to Survive the Alcoholic Fermentation. The Lactic Bacteria.—These, of which there appear to be several species, are anaërobic rod-shaped organisms, much resembling *B. subtilis* in appearance, and are often found in beer which has turned sour. They were first described by Pasteur. One of these has been specially studied by Van Laer, who named it *Saccharobacillus Pastorianus*. It is able to grow both in wort and beer, the latter it first renders turbid, then acid, and at the same time communicates an unpleasant odour and flavour to it. This bacterium ferments some of the maltose and dextrin of the wort, yielding lactic, formic, and acetic acids, together with ethyl alcohol and some of its higher homologues. Another probably different species has been isolated by Lindner from Berlin "Weiss Bier," and another by Hayduck from distillery mash. The development of the lactic ferments is either partially or completely arrested by the bactericidal action of the soft resins of the hop; consequently they are most likely to appear in lightly hopped beers.

The Acetic Bacteria.—These organisms, which occasionally make their appearance in beer, are not nearly so sensitive to the antiseptic influence of the hop as the lactic ferments. Their presence may be recognised by the vinegar-like odour which they communicate to the beer. Being highly aërobic organisms, they cannot develop to any extent without a liberal supply of air. The acetic ferments have been very thoroughly investigated by Hansen,¹ who was able to isolate three varieties, which he respectively named *Bacterium aceti*, *B. Pastorianum*, and *B. Kützingianum* (Figs. 39, 40 and 41). When cultivated in beer, at a temperature of 34° C. (93° F.), they all form pellicles on its surface, which differ markedly in appearance. Beers infected with any of the three bacteria remain clear at the cultivation temperature; also in the case of the two former, after the beers have been cooled down to the ordinary temperature, those infected with the last bacterium become turbid on cooling. All three bacteria assume very varied

increases to such a degree that the vessel containing it may almost be inverted without the beer running out. On introducing and withdrawing a rod, the portion of beer which remains attached to the rod is drawn out into a long string.

¹ *Compt. Rend. Lab. Carlsberg*, 1894, p. 182.

involution forms, the shapes being influenced to a great extent by the temperature at which the bacteria are grown, as may be seen from the accompanying figures. When portions of the several films are placed under the microscope and treated with iodine solution, the jelly-like substance in which the *B. aceti* are imbedded is simply stained yellow; that of the other two is coloured blue. Zeidler¹ has described two acetic bacteria which he

FIG. 39.—*Bacterium aceti* (Hansen).

FIG. 40.—*Bacterium Pastorianum* (Hansen).

distinguishes as *B. aceti albumosum* and *B. aceti friabile*. The former is probably identical with Hansen's *B. aceti*, but the second does not seem to resemble any of Hansen's varieties. Both are coloured yellow by iodine. The former has a strong tendency to produce involution forms, though the latter yields them much less readily. Both forms, when cultivated in hopped wort, form pellicles, that of the former being firm, tough, mucilaginous, coherent, and not easily broken to pieces on shaking the vessel; that of the latter is of a white, loose, flocculent nature, readily breaking to pieces on the slightest movement. When *B. aceti albumosum* is added to wort, either previous to or during fermentation, or to finished beer, it causes at first turbidity; after the beer has been kept for some time it becomes acid, and assumes a viscid condition, much deposit of a tough mucilaginous nature being formed. Similarly, liquids infected with *B. aceti friabile* exhibit turbidity, but not to such a marked extent; less acid is formed, and there is an entire absence of viscosity.

FIG. 41.—*Bacterium Kützingianum* (Hansen).

The Sarcinæ.—This name was first applied by Goodsir to a certain organism occasionally met with in the human stomach; in more recent times Hansen found similarly shaped organisms in beer wort. These latter have been investigated by Lindner, who divided them into three species. The first of these grow by division in two planes, and are termed *Pediococci*; four varieties are described, *P. cerevisiæ*, *P. acidilactici*, *P. albus*, and *P. viscosus*. The second species includes the *Sarcina candida*, *S. rosea*, and *S. aurantiaca*, which usually increase by division in three planes. The last species, which

¹ *Woch. f. Brau.*, 1890, p. 1213.

form the true *Sarcinæ*, invariably grow by the last method of division. Three species of these are described, *Sarcina flava*, *S. lutea*, and *S. maxima*. Film-formation has been observed with *P. cerevisiæ* and *S. aurantiaca*; and all, with the exception of *S. maxima* (which has not been studied), produce lactic acid, *P. acidi lactici* being the most energetic in this respect. *P. viscosus* produces at times ropiness in beer. Another species, *P. sarcinæformis*, which closely resembles *P. cerevisiæ* in appearance, has been described by Richard.¹ It produces lactic acid in considerable quantity, and also small quantities of alcohol; it may also cause the beer to become turbid and to acquire an unpleasant flavour. This, the *P. cerevisiæ*, and *P. viscosus* are regarded by Lindner as the dangerous species; and it is asserted by Lasché that *S. flava*, *S. aurantiaca*, and *S. alba* have caused trouble in American breweries. The former three varieties, when cultivated on meat-broth gelatin, form colourless colonies, which grow very slowly, and do not liquefy the gelatin. The other varieties either grow much more luxuriantly or form coloured colonies. The *sarcinæ* are motile organisms, though it is not known with absolute certainty whether they possess flagella or not; from the lively movements of some of them, it is extremely probable that they do.

THE MOULD FUNGI.

A moist organic substance of almost any kind, such as bread, boiled potato, jam, fruit, damp grains, damp barley, &c., when exposed to the air for a time, becomes covered, sooner or later, by the fluffy silky coating, often of various colours, which is familiarly known as "mould." Such layers consist, in reality, of a number of microscopical plants, which are of an exceedingly simple structure, and which, as they do not contain chlorophyll, are included amongst the fungi.

Mucor mucedo.—This mould makes its appearance on many moist organic substances when they are left exposed to the air. It is, however, most readily obtained by placing a few balls of fresh horse-manure on a sheet of wet blotting-paper under a bell-jar, and keeping the whole in a warm place for a few days. The surface of each ball will then usually be found to be covered with a luxuriant vegetation, consisting of long delicate silky filaments (Fig. 42, *a, a*), each of which ends in a rounded head that gives it the appearance of a pin. The body of the plant consists of a number of branched filaments (*b, b, b, b*), or "hyphæ," as they are called. Some of these ramify into the substratum on which the fungus grows, and the mass so formed is termed a "mycelium." The mycelia of contiguous plants interlace with each other and form a felted mass. The whole of the plant, notwithstanding its complex configuration, consists, until the period of spore formation, of a single cell, formed externally by a thin, much-branched tube of elastic, transparent, and colourless cellulose. The inside of this tube is more or less

¹ *Zeit. f. Brau.*, 1894, p. 257.

completely filled with protoplasm permeated by the cell-juice, and this latter, collecting at various points, forms vacuoles.

Growth.—The appearance of the spores and of their growth during germination can be best studied in a hanging-drop culture. For this purpose one of the ripe little round heads (Fig. 42, *c*), called “sporangia,” is detached from the plant with a platinum wire and immersed in a little sterilised

FIG. 42.—*Mucor mucedo* (after Brefeld and Kny).

a, *a*. aerial hyphæ; *b*, *b*. branched hyphæ (mycelium); *c*. sporangium;
d. columella; *e*. spores; *f*. columella; *g*. cell-fusion; *h*. ruptured zygospore;
i. formation of sporangium.

water contained in a watch-glass, and if the sporangium does not rupture spontaneously, it is crushed with the wire. A small quantity of sterilised wort is then added to the contents of the watch-glass, the whole covered up, and allowed to remain at rest for half an hour, after which the liquid is vigorously stirred up, so as to distribute the spores evenly. From this one or more hanging-drop cultures are made, taking care that each drop does not contain more than one or two spores. The culture is then placed under the microscope, and its progress watched from hour to hour. By the time the preparation has been completed, each spore will have swollen to about ten times its original size, become circular, and, as the result of active endosmosis, developed a large vacuole. In the course of a very few hours it will push out one or more buds, which gradually increase in length and

become hyphæ. As the plant absorbs more nutritive material than is required to supply the energy necessary for the performance of its vital functions, an accumulation of substance takes place in its interior, and space is provided for this first by the hypha increasing in length, and afterwards by its throwing out side-branches. When the lengthening process has proceeded for some time, the cellulose at some point on the side of the tube becomes softened, and is pushed out by the protoplasm. In this way a bud appears, which gradually enlarges to form a hyphal branch. This growth and subdivision of the hyphæ goes on until the many-branched mycelium is formed. In old plants there is a tendency for the hyphæ to become divided into distinct cells by the formation of partition walls, as is the normal course with many other mould fungi. As the hyphæ grow the protoplasm secretes new layers of cellulose, so that the cell-wall is kept intact and of about the same thickness.

Sporulation.—When the plant has attained a certain age it puts forth, at various points, upright hyphæ, somewhat thicker than those that form the body of the plant. These are called the “aërial hyphæ” (Fig. 42, *a*, *a*), and their function is to bear the organs of fructification. When each of them has attained a certain length it ceases to grow, and its upper end becomes swollen into a small knob, the “sporangium,” on which minute drops of water often appear.¹ A partition now commences to form at the junction of this knob with the stem, the end of which assumes the form of a little knob situated within the larger (*d*). A portion of protoplasm is in this way cut off from the rest of the plant, which, notwithstanding its complicated figure, had up to this time consisted of a single cell. The exterior of the sporangium becomes covered over with minute spicules of calcium oxalate, which give it a rough appearance. The protoplasm in the sporangium now becomes marked out into a number of oval masses, which, though close together, do not actually touch. These eventually become invested with a cellulose membrane, and gradually develop into perfect spores (*e*). The remainder of the protoplasm which is not used in forming the spores is then transformed into a gelatinous substance which has a powerful attraction for water. The sporangium, which in its early stages is of a pale colour, gradually, as it ripens, turns first brown, then black, its outer wall at the same time becoming very thin and brittle. When the sporangium is ripe, the contained gelatinous substance absorbs moisture, swells up, and the cell-wall suddenly bursts, causing a miniature explosion. This scatters the spores in all directions, and they may then be carried by currents of air to considerable distances. The interior knob, or “columella” (Fig. 42, *f*), as it is called, remains for some time attached to the end of the aërial hypha, often with a portion of the covering of the sporangium attached, which forms a little collar round its base. The detached spores now travel until chance brings them in contact with a medium favourable for their nutrition, when they germinate and form new plants.

¹ This is not condensed on the outside of the plant, but actually pushed through the cell-wall by the osmotic pressure inside.

Sexual Reproduction.—This is a method of reproduction which is universal in the higher forms of plant life, and it has been observed in certain of the lower members of the vegetable kingdom, among others the mucors. Two cells are always concerned in this process, one representing the male element, the other the female; hence it is called “sexual.” The mutor, so long as it grows on the surface of a liquid and has a plentiful supply of nourishment at its command, only reproduces itself in the asexual manner just described; but when it grows on a solid substratum, as is almost invariably the case in its natural state, it then occasionally reproduces itself in an entirely different manner. When this is about to take place, two short club-shaped branches grow out from contiguous hyphæ; these become filled with protoplasm, and gradually approach one another until their extremities touch. A cell-wall, which cuts off a portion of protoplasm from that of the branch, is now developed in the end of each of these short branches. Those portions of the cell-walls that are in contact then dissolve, and the contents of the two cells fuse together (Fig. 42, *g*), after which they grow into an enlarged cell called a “zygospore”; this, as it ripens, turns black, and its exterior becomes covered with warty excrescences. The zygospore is then detached from the rest of the plant, and when placed in a suitable medium, after the lapse of a period of six or eight weeks,¹ commences to germinate. On germination the cell-wall of the zygospore (Fig. 42, *h*) ruptures, and an unbranched hypha grows out from it; this sends up an upright stem into the air, at the extremity of which a sporangium is formed (Fig. 42, *j*), the spores of which when ripe escape, and reproduce the plant in the asexual manner.

Nutrition.—The tissues of the mucors and of the other mould fungi, when analysed, are found to consist of the elements carbon, oxygen, hydrogen, nitrogen, sulphur, and phosphorus, together with minute quantities of potassium, magnesium, and calcium. In order that the plants may carry on their vital functions perfectly, these elements must be supplied them in the form of such chemical compounds as they can assimilate. As the mucors possess no chlorophyll, carbon must be presented to them in the form of sugar or some analogous carbohydrate; hence they are dependent on chlorophyll-bearing plants for their carbonaceous diet. Nitrogen may be supplied to them in many forms, such, for instance, as potassium nitrate, or an ammonium salt, such as ammonium tartrate; but they can assimilate amides, peptones, and even crude proteïds. The sulphur, phosphorus, and mineral bases are best supplied in the form of the phosphates and sulphates of the metals potassium

¹ This peculiar character of not being able to germinate until after the lapse of a certain time has led to the employment of the term “resting spores,” oftener applied to the spores of the bacteria. It seems to be a provision of nature to tide the plant over a period of scarcity of food, for resting spores are, as a rule, only formed when the supply of food runs short. Analogous instances of a resting period are found in the higher plants; for instance, the tubers of the potato, the bulbs of the onion, hyacinth, and many other plants, remain perfectly quiescent during the winter months, and only begin to show signs of life in the spring, though the conditions as to warmth, moisture, &c., under which they have been kept, may not have materially differed during the two periods.

magnesium, and calcium. The following forms an excellent medium for their cultivation ;

	Grammes.
Cane-sugar	15.00
Ammonium nitrate	1.00
Potassium phosphate	0.50
Calcium phosphate	0.05
Magnesium sulphate	0.25
Water	100.00

If it is attempted to grow a mould fungus in a solution from which any one of the elements forming compounds is withheld, though it may contain all the rest, the plant sooner or later perishes and dies.

Respiration.—The mould fungi respire freely, taking in oxygen and giving out carbon dioxide ; consequently, unless liberally supplied with air, they soon perish.

Mucor racemosus.—This fungus is distinguished from *mucedo* by its much smaller size, by the upright hyphæ (which are short and bear mouse-grey

FIG. 43.—Gemmæ of *Mucor*.

sporangia) being branched, and by the columella, which is round or pear-shaped. The external covering of the sporangium is tough and resistant, and is not soluble in water. As regards respiration, nutrition, and reproduction it resembles *mucedo*. It has, however, a third method of reproduction which, so far, has not been observed in all the other members of the family ; this is by the formation of gemmæ. When this process is about to take place, the protoplasm in some of the hyphæ of the mycelium collects at certain points in the tube ; transverse walls then form, which divide these portions from the rest of the contents of the tube and convert them into distinct cells, in the interior of which fatty substances are deposited. The cell-walls then thicken considerably, the intermediate portions of the hyphæ decay, and the gemmæ become detached ; each of these under favourable circumstances can develop into a new plant. But the most curious fact with regard to these gemmæ is their power of setting up alcoholic fermentation when they are compelled to grow *under the surface* of a saccharine fluid. When cultivated under these conditions they throw out buds of a spherical or oval shape, which often hang

together for a time in strings or clusters (Fig. 43). In this form they bear such a strong resemblance to yeast that they have been called "mucor yeast," and this resemblance has often led observers to erroneously conclude that they had been able to transform the mucors into yeast.

Other Mucors.—In addition to these two, several other members of the family are of frequent occurrence. The *Mucor stoloniferus*, so called from the stolons or runners which it throws out, something after the fashion of the strawberry plant: these bend down towards the substratum on which the plant is growing, and when they touch it take root again, as it were, and form

FIG. 44.—*Mucor circinelloides* (after Van Tieghem and Gayon).

new plants. Several aerial hyphae arise at one point in the substratum, each bearing a black sporangium. The columella has the shape of half a sphere. In *Mucor circinelloides* (Fig. 44) a remarkable branching of the aerial hyphae takes place, and the small branchlets which bear the sporangia become considerably curved during development; hence the name of the plant. Like *racemosus*, it forms gemmæ. *Mucor spinosus* has chocolate-brown sporangia, and the upper portion of its columella is studded with pointed excrescences. *Mucor erectus* resembles *Mucor racemosus* very much in form, but has, like *spinosus*, spicules on the top of its columella.

Alcoholic Fermentation.—As has been incidentally mentioned, some of the *Mucor* family have the property of exciting alcoholic fermentation. The *Mucor mucedo* does not form gemmæ, but its mycelium when submerged in wort is able to excite a feeble alcoholic fermentation, yielding, according to Hansen, in two months and three-quarters 1 per cent. by volume of alcohol, and in six months 3 per cent. The power of causing fermentation, however, seems to be greater in those mucors which form gemmæ; thus *Mucor erectus*, when grown in the submerged condition, yields, in a wort of a specific gravity of from 1056 to 1060, as much as 8 per cent. of alcohol by volume; it can also ferment a solution of dextrin. The fungus secretes an enzyme which is capable of hydrolysing starch. *Mucor spinosus*, under similar circumstances, yields 5.5 per cent. by volume of alcohol; *racemosus*, 7 per cent. This last secretes an enzyme capable of inverting cane-sugar, and is the only member of the family which can ferment this sugar. *Mucor circinelloides*, though unable to ferment cane-sugar, rapidly attacks this sugar after inversion.

SIMPLE MULTICELLULAR ORGANISMS.

Penicillium glaucum.—This mould is of almost universal occurrence ; it is met with on jam, cheese, bread, decaying fruit, &c. In the malt-house it frequently makes its appearance on the barley when on the floor, and is especially prone to attack damaged corns. In warm weather it grows with enormous rapidity. In the brewery it is often found on the walls and ceiling of the various rooms, on the sides of vats, the outside of casks, also on the insides of such as are left open to the air, and in any other place where masses of damp organic matter, such as portions of spilled grains, yeast, &c., collect. Its green-blue colour is due to the myriads of spores, which form with the most extraordinary rapidity.

Growth.—If a little of this greenish-blue powder is taken up on the end of a platinum wire, stirred into a drop of wort and a hanging-drop culture made from this, the spores may be examined and the subsequent development of the plant studied under the microscope. The spores (Fig. 45, *a*) are minute spherical bodies, about 2.5 micromillimetres in diameter, which when seen singly are colourless ; it is only when viewed in mass that they exhibit their peculiar colour. Each spore consists (Fig. 45, *a*) of a thin cell-wall filled with protoplasm, which, when the spore comes in contact with water, rapidly absorbs moisture, the spore swelling out to about three times its original bulk. It then commences to germinate ; little projections make their appearance at one or more points (Fig. 45, *b*) in the cell-wall, and these gradually develop into hyphæ (Fig. 45, *c*), which do not extend to any great length before transverse partitions make their appearance. These divide the hyphæ into a number of distinct cells, and in this way convert the plant into a multicellular organism (*d*). Many of the hyphæ, after having become divided into segments in this way, throw out a side-bud, which is generally situated immediately behind a partition-wall. These buds increase in size and become hyphæ, which extend in length, and then form branches in a similar manner. The tips of many of the short branches meet and fuse together, and in this way a true network is formed ; these frequent branchings and fusions lead at last to the formation of a dense, complicated, felted mycelium. The mycelia of contiguous plants closely interlace, and often form a mass of considerable size on the surface of the liquid on which the fungus grows, of a nature so tough and coherent that its removal from the flask or bottle in which it has grown is often a matter of considerable difficulty. The plant throws up erect filaments, or aerial hyphæ, which bear the spores. When each of these has attained a certain length it ceases to grow, and the cell at its apex throws out a bud which enlarges to a cell. On the top of this another cell (Fig. 45, *e*) develops, which swells at its apex, and just below the swelling a constriction forms. The portion thus marked off becomes rounded and forms the first spore. The cell again swells, another portion is similarly cut off, and the second spore produced, the process being repeated until a string

of spores resembling a chaplet of beads is formed. In the meantime side-cells, which form strings of spores in a similar manner, are put out from the cell at

FIG. 45.—*Penicillium glaucum* (after Brefeld and Zopf).

a. spores ; *b.* and *c.* incipient hyphae ; *d.* multicellular hyphae ; *e.* hypha forming spores ; *f.* fully-formed head ; *g.* conjugation of hyphae ; *h.* sporocarp (seen in section).

its apex, and in this way the brush-like arrangement (Fig. 45, *f*) is produced. The intermediate portions which connect the spores then decay, and leave the spores free to be wafted away by the slightest current of air. The spores are covered externally by a fatty layer, and are, conse

quently, not readily wetted; when a drop of water falls on a mass of them it simply rolls off.

Sexual Reproduction.—In addition to the asexual method of reproduction, the *Penicillium* can also propagate itself sexually when placed under certain conditions, the chief one being apparently the absence of a sufficient supply of oxygen. Brefeld effected sexual reproduction by placing the mycelium of the *Penicillium* between slices of bread, which were then kept in a dark, damp place, access of air being excluded. Lindner has observed the process take place in a gelatin-wort cultivation in a Petri-dish from which air was excluded. When sexual reproduction is about to take place, certain short branches of the hyphæ twist themselves together into a sort of double spiral (Fig. 45, *g*), and as it is exceedingly probable that the protoplasmic contents of each couple mingle together, it is considered that one hyphæ represents the male element, the other the female.

A series of remarkable changes now take place, which end in the formation of a small round body, the "sporocarp" (Fig. 45, *h*), which passes several weeks in a resting condition. If, at the end of this period, the sporocarp is placed in a damp situation, it takes on a series of changes, at the termination of which its interior is filled with an enormous number of spores, together with crystals of calcium oxalate, which latter salt also collects in the cell-wall and renders it extremely brittle. When this ruptures the spores are set free. The whole process is exceedingly slow, requiring for its completion six or eight months. Each of the spores produced in this manner is somewhat spindle-shaped in form, with three or four ridges on its external covering, and a circular groove running round its margin. The sexual spores when placed under suitable conditions germinate at once, but if kept in a dry condition in the unruptured sporocarp they preserve their vitality for several years.

Respiration.—The *Penicillium* respire, that is, it takes in oxygen and gives out carbon dioxide. That it requires a considerable amount of this gas may be seen by watching a hanging-drop culture from day to day; the plant being in an air-tight cavity, soon begins to languish, and finally perishes from want of air.

Nutrition.—The fungus is by no means choice in the matter of diet. Since it contains no chlorophyll, it is unable to obtain its supply of carbon from the carbon dioxide of the atmosphere, but it is able to appropriate the carbon of a vast number of other substances, such as the various sugars, mannitol, glycerin, acetic, tartaric, citric, or succinic acids, asparagine, leucine, alcohol, and many other compounds. When grown on a solution of racemic acid, which is composed of equal molecules of the dextro- and lævo-tartaric acids the *Penicillium* feeds upon the dextro variety and leaves the other almost intact. This property was discovered by Pasteur, who utilised it for obtaining lævo-tartaric acid. The *Penicillium* exhibits an equal indifference as to the source of its nitrogenous supply. It obtains it most readily from a peptone, but it can derive its nitrogen from almost any ammonium salt, such as the carbonate, lactate, tartrate, or phosphate; from ethylamine, propylamine,

trimethylamine, or from the amides—acetamide, oxamide, asparagine, leucine &c. It is able to obtain its supply of sulphur not only from sulphates, but also from sulphites, hyposulphites, and from proteïds. The organism produces oxalic acid during its growth, and this often combines with calcium to form crystals which are deposited in the solution on which the plant grows. Occasionally the oxalic acid combines with potassium, and in this case the resulting salt remains in solution. Calcium oxalate is very frequently met with in plant life; Sachs considers that it is secreted in order that the plant may be able to decompose calcium sulphate, and in this way obtain the supply of sulphur that it requires for the formation of its proteïd constituents.

Penicillium when grown submerged in a saccharine fluid is unable to excite alcoholic fermentation. When placed under these conditions it ceases to grow normally, its hyphæ assume peculiar and irregular forms, and large quantities of fat become deposited in their protoplasmic contents; indeed, in such cases the fat has been found to amount to three times as much as that which the plant contains when grown under normal conditions.

Temperature.—*Penicillium* is able to grow within very wide limits of temperature. Its spores will germinate at any temperature between 2° and 43° C (35° and 110° F.), but 22° to 26° C. (72° to 79° F.) seems to be the most favourable temperature. Pasteur found that the dry spores could withstand a temperature of 108° C. (226° F.) without losing their vitality, but that they were soon killed when immersed in boiling water.

Eurotium aspergillus glaucus.—This exceedingly common mould fungus (Fig. 46) is met with under much the same conditions as the *Penicillium glaucum*. It closely resembles this latter in the shape of its spores, their germination, and the subsequent formation of a mycelium therefrom; the principal difference observable being that the partition-walls of its hyphæ are not so numerous, and consequently the cells are somewhat longer.

The growth is best studied in a hanging-drop culture. When a certain amount of mycelium has formed, the plant begins to throw out upright stems which contain very few partition-walls, and these eventually bear the spores.

Aërial Sporulation.—The method of fructification, however, differs considerably from that of the *Penicillium*. Each upright hypha, after having attained a certain length, ceases to grow, and its upper extremity expands into a pear-shaped head (Fig. 46, a). All over the surface of this, little protuberances, like so many pegs, arise, the protoplasm of each of these being continuous with that of the head. The end of each of these pegs then puts forth a little round bud, under which a constriction forms, and this gradually tightens until a little round knob is formed hanging by an exceedingly delicate neck. The end of the peg then similarly throws out another bud, the process of constriction is repeated, and this goes on again and again until a chaplet of cells resembling a string of beads is produced, of which the oldest are the most distant from, the youngest nearest to, the head. These are the spores, and when the growth of a head is completed, these numerous chains of spores have the appearance of a brush-like ball (Fig. 46, b). The head, when all its proto-

plasm has been exhausted, gradually decays, and the spores fall off; but those which have ripened before this takes place, may be removed by the slightest touch or by a slight breeze; they then float in the air, and may be widely disseminated by air-currents. The plant, in the earliest stages of its growth, consists of a white patch of mycelium, which gradually turns grey; it is only after the spores are formed that it assumes a green appearance. Each spore as seen individually under the microscope is colourless, and it is only when a large number are seen together, as when growing on the plant, that they exhibit their peculiar green colour.

Sexual Reproduction.—In addition to this asexual method of reproduction the *Eurotium* can produce an entirely different set of spores by a sexual

FIG. 46.—*Eurotium aspergillus glaucus* (after De Bary).

a. hypha commencing to form spores; b. fully-formed head (seen in section);
c. conjugating hyphæ; d. sporocarp.

process. When this is about to take place, two of the short hyphæ coil and intertwine together (Fig. 46, c), and their protoplasmic contents become fused together. A complicated series of changes then ensues, which finally terminates in the formation of a spheroidal body called the "sporocarp" (d), of a yellow colour, which contains a host of spores, each of which is in shape something like two watch-glasses joined by an enveloping band. When placed in a suitable medium, the protoplasm of the spore swells up, pushes aside the two concave valves, and throws out a bud. This gradually enlarges, forms partition-walls, and becomes a hypha, which grows and eventually forms a mycelium. From this the upright stems for bearing the asexual spores arise, whilst sporocarps may also form in other portions of the mycelium.

Respiration and Nutrition.—These processes are carried on in a manner analogous to those of the *Penicillium*, though the *Eurotium* is unable to

derive its supply of food from so large a number of substances as the former.

Temperature.—The *Eurotium* grows best at a temperature of from 10° to 15° C. (50° to 59° F.), and is said to be unable to withstand a higher temperature than 30° C. (86° F.). This question has, however, been very incompletely studied.

Botrytis cinerea.—This is another fungus commonly found growing on decaying organic matter, in the form of small greyish yellow patches, from which aerial hyphæ of the length of about a millimetre ($\frac{1}{25}$ of an inch) arise, each of which bears a tufted head on which are seated a mass of spores (Fig. 47, *a*). If a few of these are removed and placed in a hanging-drop culture, their appearance and subsequent development may be observed in the usual way.

FIG. 47.—*Botrytis cinerea* (after De Bary).

- a*, tufted head of hypha with spores; *b*, germinating hypha; *c*, hypha with bunched spores; *d*, sclerotia; *e*, cup-shaped sclerotia; *f*, ascospore.

Growth.—The spores germinate (Fig. 47, *b*), and form a dense network of mycelium, similar to that of the *Penicillium* or *Eurotium*. In a few days the cell-walls of the hyphæ of the mycelium become swollen and gelatinous, and, when lifted out of the liquid in which the plant is growing, appear glairy and feel slippery, as if they were coated with mucilage. When the mycelium is some days old, some of the hyphæ, which in the course of their growth have come in contact with a solid body, such as the walls of the

vessel the plants are grown in, rapidly throw out a number of small branches, which subdivide, and at last assume the form of little tassels, which gradually turn black. These develop a sort of mucilaginous matter by means of which they become firmly adherent to any object with which they come in contact.

Aërial Sporulation.—From the mycelium, which gradually assumes a greyish-brown colour, upright hyphæ (which are divided by septa and often branch) arise; these are destined to bear the spores. After these aerial hyphæ have grown to the height of a millimetre, they throw out stout, short branches nearly at right angles to the principal stem, and each branch subdivides again into several shorter twigs. The ends of each of these swell, and on its swollen surfaces an egg-shaped spore gradually develops. The spores, when ripe, are about 8 to 9 micromillimetres long and 6 to 7 broad, pale reddish-brown in colour, and connected to the stem by a delicate neck, only one spore being formed at the end of each twig. When growth is completed the head of spores somewhat resembles a bunch of grapes

(Fig. 47, *c*); hence the name of the plant, *Botrytis*, from the Greek word signifying such a bunch.

Sexual Reproduction.—When this takes place in the *Botrytis*, round or irregular shaped, hard black bodies, called “sclerotia” (Fig. 47, *d*), form in the mycelium. The size of these varies from half a millimetre to several millimetres in diameter; they are made up of an extremely dense and complicated mass of interlacing hyphæ. If, directly after their formation, they are removed from the plant and placed in a nutrient fluid, or on a nutrient substratum, they at once commence to throw out upright hyphæ which bear spores. If, however, they are dried, kept for at least a year, and then buried under the surface of soil which is constantly kept moist, small buds protrude from their surfaces. Each of these grows into a stout, hairy, cylindrical stem (*e, e*), which at length pushes its tip through the soil and then ceases to grow. The apex of each of these stems expands into a little cup-shaped formation, the interior of which eventually becomes lined with a velvety layer, each strand of which is an elongated cell. Some of these cells remain barren; in others the nucleus divides, first into two, then into four, then into eight (*f*); each of these divisions of the nucleus attaches to itself a portion of protoplasm, and after investing itself with a cell-wall, finally becomes an “ascospore.”¹ The elongated cells in which the ascospores have formed absorb water, and the effect of this is gradually to push the spores towards the upper extremity of the cell; when the pressure becomes sufficiently great the end of the cell ruptures, and the spores are driven out with an amount of force sufficient to carry them to some distance. The spores, if they happen to alight in a suitable medium, germinate in a few hours, develop into a mycelium which eventually throws out spore-bearing aërial hyphæ and forms sclerotia.

Nutrition.—The plant will grow on almost any kind of organic substance, in presence of moisture and oxygen, provided the temperature is a few degrees above the freezing-point. It is able to obtain its supply of carbonaceous diet from the sugars, or from organic acids, such as tartaric, citric, &c. Its tissues, upon analysis, are found to contain large quantities of potassium phosphate; the plant, during its growth, produces much oxalic acid, and this combines with potassium. It secretes two enzymes; one of which dissolves cellulose and the other inverts cane-sugar; but it is quite unable to excite alcoholic fermentation.

The *Botrytis* also grows on living plants, its hyphæ piercing the cuticle and penetrating into their cells; in this way it often causes considerable damage to fruit and other crops. The peculiar flavour and bouquet of certain Rhine wines are caused by modifications which this fungus induces in the contents of the over-ripe grapes before their juice is subjected to the vinous fermentation. *Botrytis* is sometimes the cause of a smoky flavour in wines, and it is probable that a similar flavour occasionally met with in beer is also produced through its agency.

¹ So called from being formed inside a cell or sack (*ascus*, a sack).

Aspergillus oryzae.—A fermented liquor known by the name of “saki,” has long been made in Japan from rice by the agency of this fungus. It is manufactured in the following manner: A quantity of rice is first steamed, allowed to become cold, and a portion of rice grains, kept over from a previous brewing and on which the mycelium of the fungus is growing, is mixed with it. In a few days the mycelium will have spread over the new rice, this is then mixed with a further portion of steamed rice, the process being repeated until a sufficient quantity of infected rice is obtained. Under this treatment the mass of rice develops an odour similar to that of apples or pine-apples. In the actual brewing 21 parts of this infected rice are mixed with 68 parts of steamed rice and 72 parts of water, and the semi-solid mass thus obtained is kept at a temperature of 20° C. (68° F.) for several days, at the expiration of which it becomes considerably liquefied, the starch being hydrolysed by an enzyme secreted by the fungus. In about three days alcoholic fermentation sets in spontaneously, and lasts for about three weeks. A beverage is obtained in this way which, after filtration, is of a bright, sherry-wine colour, and contains from 13 to 14 per cent. of alcohol. The Koji-ferment has been much studied; it has been found to contain various enzymes, all of which are able to hydrolyse starch, dextrin, maltose, and cane-sugar. Hydrolysis of the proteïds also appears to take place, for much insoluble proteïd matter is converted into soluble during the process.

Juhler, who investigated the action of the Koji-ferments, was led to the conclusion that the alcoholic fermentation is brought about by the ripe spores of the aspergillus. These, when grown at a suitable temperature and with a free supply of air, develop a mycelium in the ordinary manner, but when, after having liquefied the starch, they sank into the liquid, and were excluded from contact with the air, Juhler thought that he had been able to observe a series of extraordinary changes, which ended in their conversion into *Saccharomycetes* or true yeast. Recent investigations have, however, disproved the correctness of these conclusions.

FIG. 48.—*Chalara mycoderma* (after Hansen).

In addition to the *Aspergillus oryzae* two other fungi have been found in the Koji-ferment, a white mould very similar to that described by Calmette as *Amylomyces Rouxii*, and a variety of *Mucor*, but these are not such energetic agents in effecting the hydrolysis of starch as the *Aspergillus*.

Chalara mycoderma.—This is another mould fungus which is able to grow on wort or beer, on which it forms a greyish film, which consists of a branched mycelium (Fig. 48). The contents of some of its hyphæ have a brilliant refractive appearance, and from these conidia of different shapes and sizes arise. The fungus is met with very abundantly on the surface of grapes.

Oidium lactis.—This fungus (Fig. 49) is often found on the surface of milk, hence its name. It is also often met with in air analyses, where it forms on the thin gelatin layer a bright, silky mass of mycelium, the hyphæ of which radiate outwards from a common centre. Grown on a thick layer of gelatin, it forms large colonies having a radiating form, and a dry, powdery appearance on their surface. A considerable quantity of gas is liberated by each colony, which forces up the gelatin into a bubble; this may become as large as a walnut, but does not burst. The fungus is met with on malt,

FIG. 49.—*Oidium lactis* (after Hansen).

on leaky places in brewing vessels, and is sometimes found growing on yeast. When vegetating on wort it forms white, floating masses having a woolly appearance, under which bubbles of carbon dioxide collect. The woolly mass consists of transparent, branched, forked and thin-walled hyphæ, the extremities of which are divided into separate cells by numerous transverse septa. These cells form the spores of the plant; they finally become detached, and, when placed in conditions favourable to their growth, germinate. The fungus is not, as was at one time supposed, the cause of the lactic fermentation in milk; it can, however, excite a feeble alcoholic fermentation in wort. It grows freely on yeast, and though the organism is not known to exercise any prejudicial influence on either yeast or beer, yet its presence

there is undesirable. When the spores, which consist of short cylinders, are cultivated in a hanging-drop, they swell up, become egg-shaped, and then throw out one or more hyphæ, which grow rapidly and branch freely. Numerous partition-walls then appear, and the separate cells formed in this way break off with a kind of convulsive movement, which can often be induced by tapping the stage of the microscope.

Oidium lupuli.—Under this name Matthews and Lott have described a fungus somewhat akin to the *Oidium lactis*, which they found growing on spent hops in the form of a reddish-brown or salmon-coloured dust. When cultivated in a hanging-drop it forms a luxuriant aërial mycelium, the hyphæ of which become branched at their apices. Each branch then divides up by the formation of transverse septa into individual cells which constitute the spores of the plant.

Lindner describes another *Oidium*, provisionally named *pullulans*, which he has often found in air analyses, and in samples of lager beer removed from the storage casks by spiling. It is able to grow on the surface of wort, but does not seem capable of exciting alcoholic fermentation. Its influence on beer is unknown.

Dematium pullulans (Fig. 50).—Another fungus also frequently met with on the surface of grapes and other fruits. It is frequently found in

FIG. 50.—*Dematium pullulans*
(after Loew).

air analyses, and its presence can be readily detected by the rapidity with which it liquefies the gelatin layer. According to Lindner, who has studied the habits of this fungus, it grows rapidly in hopped wort, in which it throws off cells strongly resembling those of yeast. In a few days numerous flocculent slimy masses make their appearance under the surface of the wort, and cling tenaciously to the sides of the vessel. The liquid itself, although not rendered turbid, becomes ropy, and can be pulled out into long strings by means of a platinum loop. A microscopical examination of a drop of the wort, when it has arrived at this stage, reveals nothing but the presence of a number of isolated cells resembling long budding yeast-cells.

After some days the surface of the wort becomes covered with long threads in which the resting spores of the fungus may be seen; these, which are at first of a dirty green colour, gradually turn black. The ropiness of the liquid is caused by the deposition of a transparent mucilaginous matter on the walls of the cells, which keeps them at some considerable distance apart. During the time the wort is turning ropy it develops a paste-like flavour, and this persists after it has undergone

alcoholic fermentation. During the latter process the ropiness disappears and the *Dematium* cells die. The acidity of the beer does not seem to be influenced by an invasion of this organism. The fungus can induce a similar ropy condition in a solution of cane-sugar.

Apparent Origin of Wine Yeast.—Jørgensen, incited by the supposed discovery of Juhler that the spores of the *Aspergillus oryzae* could be transformed into yeast-cells, thought that wine yeast must have some similar origin. After much fruitless experimenting, he found, amongst the mould

FIG. 51.—*Monilia candida* (after Hansen).

fungi growing on the surface of grapes which had been kept for some time in a moist atmosphere under a bell-glass, a vegetation consisting of numerous branched hyphae, which subsequently formed conidia. On cultivating these conidia upon the surface of sterilised grapes, he thought he had observed a complicated series of changes in the vegetation thus produced, which ended in the formation of true yeast. Other observers, notably Klöcker and Schiöning, have repeated these experiments with an entirely negative result.

***Monilia candida*.**—This fungus (Fig. 51) occurs as a white layer on cow's dung, and on the surface of sweet succulent fruits. When submerged in wort it excites alcoholic fermentation, and develops cells much resembling those of yeast. It ferments maltose and cane-sugar, and has recently been

shown by Fischer, in opposition to the views previously held, to secrete invertase.

Mould Fungi Dangerous on Brewing Premises.—The presence of mould fungi, either in the malthouse or the brewery, is highly undesirable, since, as they grow and multiply with extreme rapidity, and are always accompanied by bacteria, they may at times become a source of real danger. Barley, on the growing floor, is especially liable to be attacked by *Penicillium glaucum*, *Mucor mucedo*, and *Aseprgillus glaucus*, all of which secrete diastases that convert a portion of the starchy constituents of the barley into sugar, which eventually becomes transformed into acids. In this way a portion of the malt, which should have contributed to the production of extract, is not only lost, but converted into substances of a prejudicial nature. The moulds communicate an objectionable flavour to the growing barley that is not lost on kilning and which persists in the finished beer. On rare occasions the barley becomes infested with a *Fusarium* which gives the resultant malt a reddish tint and renders it quite unsuitable for the production of beer.

Worts, especially during hot weather, may become invaded by some of the mould fungi. The smoky flavour due to the presence of *Botrytis cinerea* has already been mentioned. Beers weak in alcohol may be infested by *Monilia candida*, which, when growing under these conditions, has very much the appearance of yeast. *Oidium lactis* sometimes makes its appearance during hot weather on the surface of beer, over which it rapidly spreads as a thin skin resembling powdered starch; breweries situated in close proximity to stables are especially liable to the invasions of this fungus. Hops which have been imperfectly dried, or which are kept in a damp place, are liable to the attacks of some of the *Mucedo* species. This causes them to become heated and take on a peculiar fermentation, in which trimethylamine and butyric acid are formed, bodies that communicate an exceedingly unpleasant flavour to the hops.

As the presence of moulds in the malthouse or brewery is so highly undesirable, they should be destroyed as soon as patches of them make their appearance. A solution of chloride of lime (bleaching-powder) is a very effective mould-destroyer for those places where it can be used, but an alkaline hypochlorite solution such as the so-called "antiformin" is more convenient. For the interior of vessels, &c., a strong solution of sulphurous acid or bisulphite of lime, which acts as a powerful fungicide, may be used. A preparation sold under the name of "Antinonnin" is also a very efficient fungicide. It is a pasty mass, consisting of soap, glycerol, and potassium dinitro-cresol ($C_6H_2(NO_2)_2CH_3OK$), and is generally mixed with whitewash and applied to walls and woodwork. Its nitro-cresol constituent is an exceedingly poisonous substance.

THE HIGHER PLANTS.

These may be regarded as vast congregations of cells, all of which, at an early stage in their existence, have had the same simple structure as the yeast-cell. In this simple organism we have seen that one portion of its tissues performs one, another portion another function; thus the cell-wall, in addition to being a protective layer for the protoplasm, carries on the important function of osmosis, and the protoplasm itself performs several different functions. A certain amount of differentiation of function is therefore observable even in the structures of the simplest vegetable organisms, but, as we proceed higher in the scale of plant-life, this differentiation of function is not merely confined to the contents of the individual cell, but is extended to the cells themselves. We then find special cells set apart for the performance of certain functions, and the higher we proceed in the scale, the more complex the organisation of the plant becomes, and the greater the differentiation of function between its various groups of cells. Though the embryo of the barley-plant as it exists in the barley-corn consists of an aggregation of simple cells; yet even at this early stage of its existence there is no difficulty in recognising, from the way in which these cells are arranged, that they are already marked out for different purposes. The portion of the germ which is intended to form the root is readily distinguishable from that which is intended to form the stem. But as growth proceeds and the cells multiply by division, the cells which are concerned in the formation of the root become further subdivided into distinct groups; one of these eventually forms the epidermis or exterior covering of the root, the cells of another group coalesce to form vessels for the conveyance of water, together with those constituents of the soil which are dissolved in it, from the root to the leaves. Other cells form delicate prolongations called the "root hairs," the function of which is to absorb water from the soil. Similarly, as growth proceeds, changes take place in the cells intended to form the stem; cellulose of a tough resistant nature is deposited in the walls of those cells intended to give rigidity to the plant and enable it to preserve its shape against external influences. Other sets of cells become elongated and unite at their ends to form vessels for the transmission of the various liquids of the plant. Another series unite and form the leaves, in the exterior cells of which the chlorophyll granules are met with in the greatest abundance. When the period of flowering approaches, certain cells in the apex of the stem commence to form the complicated series of tissues which first constitute the various parts of the flower and afterwards those of the fruit. In the case of such a plant as the barley-plant, which only lives for a year, during the period of its fructification nearly the whole of the starch and other carbohydrate matter contained in the plant, with the exception of the cellulose, is conveyed to the seed and there stored up as starch. The whole of the carbon of this starch, with the exception of the minute quantity derived from the germinating seed,

has been separated from the carbon dioxide of the atmosphere by the chlorophyll granules of the plant's leaves and stem, and as these granules are most abundant in the leaves, it follows that the bulk of the carbon must have been assimilated by them, afterwards to be conveyed down the conducting tissues of the leaves in the form of sugar, then up the stem, at the apex of which it is finally deposited as starch in the seeds.

Like the *Protococcus*, the higher plants require carbon dioxide and water for their nutrition, also nitrogen, sulphur, and phosphorus for the construction of their proteid constituents, and also the mineral elements potassium, magnesium, calcium, and iron. These latter elements are derived from the nitrates, phosphates, and sulphates, which are invariably present in the soil, and which are dissolved by the water that is absorbed by the root hairs of the plant, which insert themselves for this purpose into the interstices between the particles of the soil. It is then passed by them into the root, from which it gradually ascends through the stem of the plant to its leaves. The amount of water above and beyond that required for the actual nutrition of the plant is returned to the atmosphere as vapour, leaving behind in the leaves the various salts it previously held in solution.

The Substances produced by the Cells of Different Plants.—Hitherto only those products of plant-life which are indispensable to the life of the plant have been mentioned, but very many plants form a variety of other substances from the four elements carbon, hydrogen, oxygen, and nitrogen, which are often exceedingly complex in composition, and differ with each plant. The number of the substances so formed is almost incalculable. For example, the hop plant, in addition to carrying on those functions that are absolutely necessary for ordinary plant-life, secretes the various volatile oils upon which its aromatic flavour depends, the resins and bitter principle which are of such importance to the brewer, the tannin, &c. The number of different substances which have been isolated from plants, especially of those known as medicinal, is simply enormous, and even this large number is probably but a small fraction of those which are still unknown.

Germination of Barley.—This, and the complicated series of physiological changes which accompany it, are subjects of the deepest interest to the brewer, for during the process of germination, barley, which, as such, is totally unfitted for brewing purposes, is converted into malt. The grain of barley contains, in addition to the germ or embryo, a large store of material such as starch, proteids, mineral matters, &c., which are destined to serve as nourishment to the young plant before it has thrown out its first green leaves, after which it is capable of maintaining an independent existence. This store of reserve material, as it exists in the barleycorn, is in a state quite unfitted for the nutrition of the growing embryo; the starch and a large part of the proteid matters are in an insoluble condition, and the greater portion of the proteid matter which is already soluble is in a non-diffusible form, in which condition it cannot be utilised by the young plant until after considerable modification. Everything is, however, ready for altering

this condition of things; enzymes, which are destined to induce the necessary metamorphoses, are already to some extent present in the seed, and the material is at hand for the production of those other enzymes which are necessary to complete the transformation. All that is required to set in action the vital machinery of the barleycorn is the presence of water in sufficient amount, a suitable temperature, and a supply of oxygen. When vital activity has been fully aroused, an abundance of those enzymes are secreted

FIG. 52. A—Section from a Grain of Barley.

A. The germ and its appendages.—*a*. rudimentary leaves; *b*. rudimentary stem; *c*. rudimentary root; *d*. starch cells (empty); (*e*) epithelial layer; *f*. layer of emptied cells; *g*. starch cells (filled).

whose function it is to convert the reserve materials into soluble and diffusible forms fitted for the plant's nutrition during the earliest stages of its existence. But in germinating barley for malting purposes, as soon as a certain amount of change has been secured, the maltster steps in, and, by withdrawing water from the grain, puts an end to further vital action. His object is to secure certain important modifications in the grain which are brought about by enzymic action, and also to obtain an abundance of those enzymes which convert the starch of the malt in the mash-tun into substances that are for the most part fermentable, and which transform the albuminoids into derivatives assimilable by the yeast.

Structure of the Barleycorn.—As may be seen by examining the accompanying illustration (Fig. 52, A), which represents the germ end of a grain of barley cut in two through the furrow which extends down the face of the

corn, the seed is divided into two chambers by a sort of partition-wall (*e*), the lower one containing the germ, which would, in the natural course of circumstances, develop into the barley plant. Even at this stage of its existence the

B. Section showing secretory layer of epithelium (more highly magnified).—
d, walls of starch cells; *e*, secretory layer; *f*, layer of emptied cells;
g, cells filled with starch granules; *h*, cells of the scutellum.

C. Section showing aleurone layer.—*g*, starch cells; *j*, aleurone layer; *k*, layers
 which collectively constitute the husk.

FIG. 52. B and C—Sections from a Grain of Barley.

rudimentary stem and root (*b* and *c*) are distinguishable. The germ lies embedded in a mass of cells, the uppermost layer of which consists of the series of elongated cells (*e*) termed the "secretory layer"; it has certain very important functions to fulfil during the process of germination. The endosperm is filled with a network of thin-walled cells (*A*, *g* and *B*, *g*) closely packed

with starch granules and small granules of proteïd matter. Immediately above the secretory layer are several rows of empty cells (A,*f* and B,*f*). The walls of this chamber are lined with three or four layers (C,*j*) of somewhat square-shaped cells, known as the "aleurone cells"; these, which collectively constitute the "aleurone layer," are filled with the aleurone or proteïd grains, together with small quantities of fat and mineral matter. The aleurone layer, when it reaches the scutellum or partition-wall, diminishes in thickness to a single layer of small cells, and these gradually disappear towards the base of the grain. Several layers of various other tissues surround the seed and are closely adherent to it; these collectively constitute the "husk," and are of considerable importance to the brewer, for in the mash-tun they form a mass of filtering material which completely removes suspended particles from the wort and permits it to flow off bright and clear.

Dormant Vitality.—The germ of the grain of dry barley may be said to be in a state of dormant vitality. When the barley grain is planted in the soil, it finds there all the conditions necessary for bringing its dormant state of vitality into one of activity; these are the presence of water, a suitable temperature, and free supply of air. The maltster imitates these conditions artificially; he supplies the requisite water by soaking the barley in the cistern, subsequently he keeps the grain at a suitable temperature on the floor of the malthouse, and secures its aëration by frequently turning. The barley corn, even when in a state of dormant vitality, manifests to a very small extent the actual phenomena of life; it very slowly respire, absorbing oxygen and giving off carbon dioxide, in consequence of which there is a small but appreciable loss of weight in barley during storage. It has been estimated from this cause that the best and driest barleys lose $1\frac{3}{10}$ per cent. of their weight in the first year, $\frac{9}{10}$ per cent. in the second year, and $\frac{1}{2}$ per cent. in the third year; and this loss is considerably greater with inferior and damp samples.

Germination.—When in one or the other of the above-mentioned ways the dormant vitality of the germ is roused into activity, that complicated series of processes collectively comprised under the general term of "germination" commences. Starch begins to appear in the tissues of the germ, which previously were almost or absolutely starch free, and the first rootlet begins to elongate. The cells of the scutellum, which are as yet free from starch, now show signs of activity, the proteïd matter deposited in them disappears to a considerable extent, and the protoplasmic contents of the cells assume a very granular condition and secrete much fat. As germination proceeds, the pointed mass of cells which surrounds the rudimentary root, and which constitute the root-sheath, is pushed forward by the developing root, and protrudes through the base of the grain. At this stage the barley is said to "chit." The first rootlet soon breaks through the end of the sheath, and is followed in due course by other rootlets. The cotyledonary sheath, or mass of cells surrounding the stem, commences to elongate on the third or fourth day of germination, and in so doing ruptures the proper covering of the seed, it then grows upwards between this and the husk, and forms the

acrospire or "spire" of the maltster. After growing to about fifty times its original length, its apex is broken through, and the first green leaf makes its appearance, but in the process of malting the growth of the grain is arrested long before this takes place.

First Signs of Enzymic Action.—According to Brown and Morris when the first rootlet is breaking through the root-sheath, starch begins to appear in the tissues of the germ, also in the protoplasm of those cells of the scutellum which are nearest the secretory layer, and it gradually invades the deeper-seated cells. At this period the contents of the cells of the secretory layer have assumed those appearances which indicate that they are now in a vigorous state of activity. The cellulose walls of the empty cells of the endosperm, which are situated immediately above the secretory layer become softened and partially dissolved, the dissolved matter passing into the cells of the scutellum, there to be transformed into starch. The dissolution process gradually extends to the cellulose walls of the whole of the starch-containing cells of the endosperm, and until these are affected no evidence of a solvent action can be observed in the starch grains themselves. Thus it was supposed that the first formed enzyme was solely one which dissolved cellulose, and it was consequently termed a "cytohydrolyst." As the solution of the cellulose proceeds, the tough resistant contents of the endosperm gradually become softened, and if rubbed between the finger and thumb when this stage is completed, appear and feel like a moist powder. This so-called "mealy" or modified condition the maltster endeavours to bring about to its fullest extent in the process of malting, for the degree of friability of the finished malt depends on the extent to which the cellulose has been affected. The cellulose walls of the starch-containing cells of the endosperm of different samples of barley exhibit considerable variations in their readiness to submit to enzymic influence, variations which materially depend on the influence of the soil and climate where the particular samples were grown. Those samples in which this action takes place most readily are the most suitable for malting purposes.

According to J. Grüss¹ the action which takes place on the cell walls of the barley endosperm during germination does not consist in their complete solution, as stated by Brown and Morris, but in their partial solution. Schulze has shown that these cell walls consist of two carbohydrates, an araban and a xylan; and Grüss states that the former is completely dissolved by the enzyme, whilst the latter is more or less unattacked. As a consequence of this action the cell walls become hyaline or transparent so that they cannot be seen until stained with certain dyes, and Grüss found Congo red a convenient dye for this purpose. He denotes the action which occurs on the cell walls by the term "allolysis." This limited action is sufficient to render the cell walls permeable to such enzymes as diastase. Reinitzer² concludes from his experiments that there is no special

¹ *Wochensch. Brau.*, 1896, **13**, 729.

² *Hoppe-Seyler's Zeit. f. Physiolog. Chem.*, **23**, p. 175.

cytohydrolytic enzyme secreted, but that it is the ordinary diastase which dissolves the cell-walls, and this opinion is shared by Grüss. He considers that the cellulose of which these consist is of a much less resistant nature than the ordinary hemi-celluloses, but that it is probably closely allied to them.

A. R. Ling¹ has proved that the cell wall is present in the most friable and well-modified finished malt. He finds, moreover, that a portion of the compressed layer of empty cells in contact with the epithelium of the scutellum, still remains in finished malt. As already mentioned Brown and Morris state that these cells are the first to be attacked and dissolved during germination. The two appended photographs (Figs. 53, 54) are taken from Ling's paper.

Solution of the Starch of the Endosperm.—Brown and Morris state that when the cellulose walls of the starch-containing cells are dissolved, another enzyme begins to make its appearance. This is the "diastase of secretion," so called by them to distinguish it from another diastase which is already present in the barley grain. It is through the agency of the diastase of secretion that the starch conversion in the mash-tun is effected; consequently this enzyme is a body of considerable importance. The first traces of the action of this diastase are to be found in those starch grains which are situated in close proximity to the secretory layer, and are first observed when the rootlet has grown to the length of the fiftieth of an inch, and the acrospire to about three-quarters that length. In the natural course of growth of the barley plant this solution of the starch grains proceeds until the whole of the endosperm is emptied, but in malting it is restricted within as narrow limits as is compatible with securing the proper modification of the grain. The way in which the starch granules are dissolved by the diastase of secretion entirely differs from that in which the diastase of translocation affects them. The former causes minute pittings on the surface of the granules, which increase in number and depth, and eventually give to the starch grains a very irregularly eroded appearance. A series of star-shaped fissures then appears, the layers of starch of which the grain is built up become detached, and the whole starch grain dissolves with the exception of some of the more resistant layers, which persist for some time.

Another kind of diastase exists in ungerminated barley (see p. 123), its function apparently being to effect the transportation of starch during the development of the barleycorn, while it is still attached to the plant. Hence it was termed by Brown and Morris "translocation diastase." The collection of materials which make up the endosperm are the first to be deposited in the seed, the development of the germ taking place at a later stage; and it is from the materials deposited in the endosperm that the germ obtains the matter necessary for the building up of its tissues. The empty cells that are situated immediately above the secretory layer, and which were originally filled with starch, are supposed to have been emptied for this purpose. This diastase dissolves the starch grains without previous corrosion

¹ *The Brewers' Journal*, 1904, 40, 741.

and pitting. By treating microscopical sections of germinating barleycorns with solutions of guaiacum and hydrogen peroxide, Grüss was able to

FIG. 53.

Median transverse section of finished malt corn $\times 75$.

FIG. 54.

Median longitudinal section of finished malt corn $\times 75$.

locate the diastase by observing the results it gives with those reagents.¹

¹ Pawlewski (*Ber. d. Deutsch. chem. Ges.* 1897, 30, 1313) expresses considerable doubt as to whether the reaction is so characteristic of enzymes as has hitherto been supposed.

In this way he found that in the ungerminated barleycorn the cells of the embryo, and especially those of the scutellum, contained diastase, but that it is very rarely present in the starch-containing cells of the endosperm, and then only in traces. When the grain has been steeped, the diastase is found to have left the interior of the cells and passed into their walls. As germination proceeds the presence of diastase becomes apparent in those cells of the endosperm nearest the scutellum, and in its further progress exactly follows the course of the modification in the cell-walls. Consequently, when barley is ready for the kiln, the diastase ought to have reached every cell of the endosperm, and, when sections of such grains are stained with guaiacum and hydrogen peroxide, every cell should be coloured. Also, when the sections are stained with Congo red, all the cell-walls should take this stain deeply, and so show that they are properly modified. Grüss does not agree with the view that the enzyme, which has been termed the diastase of secretion, is secreted by the germ, for he found that the separated endosperm was able to secrete diastase, and this accounts for the results obtained by Pfeffer and Hanstein (see below).

Some considerable light was thrown, during the course of Grüss's experiments, on the effect of aëration during germination. When barley was germinated in the absence of air, it was found that in eight days the diastase of translocation, though it had permeated the cell-walls of the endosperm, had been unable to effect any modification in them. But the more favourable the conditions of aëration under which the grain was grown, the more was the diastase of secretion produced, and the more was modification of the cell-walls induced. Apparently this diastase is formed by some process of oxidation.

Parasitic Nature of the Germ.—Many plants are known which, being devoid of chlorophyll, are obliged to depend upon other plants for their supply of carbohydrate food. Organisms of this kind which live on living members of the vegetable or animal kingdom are called "parasites"; the various fungi found growing on living trees are instances of these. Many fungi only vegetate on dead organic matter; these are termed "saprophytes." It has been considered that the germ of barley in the earlier stages of germination behaves like a saprophyte; Brown and Morris concluded from their experiments that the endosperm of the barleycorn was a mass of dead matter. It was found possible to remove the germs of barley and of some other seeds from their respective endosperms, and grow them on a substratum of a starch derived from another plant, or even on a layer of gelatin, moistened with a solution of such sugars as glucose, lævulose, maltose, cane-sugar, &c., this last sugar yielding the best results. The secretion of the diastase seemed to be a starvation phenomenon, for the enzyme was only secreted when the embryo was without food; when the separated germ was abundantly supplied with cane-sugar, but trifling quantities of diastase were secreted; when fed on starch, the enzyme was secreted in abundance. Endosperms, from which the germs had been removed, when kept in a moist condition, never evinced the slightest signs of vitality. (Compare, however, below.) According to this

view, the germ of barley, until it has thrown out its first leaves and is able to manufacture its supply of carbohydrate and proteid material from the constituents of the air and soil, lives the life of the saprophyte, obtaining its whole supply of nutritive material from the dead substances of the endosperm. During this portion of its existence it simply breathes, that is, absorbs oxygen from the atmosphere and gives off carbon dioxide, and its life is carried on under these conditions on the malting floor.

Some doubt has recently been thrown upon the view that the depletion of the endosperm is entirely affected through the agency of the enzymes secreted by the germ. Pfeffer and Hanstein have shown that the endosperms of barley, from which the germs had been removed before the commencement of germination, and had been replaced by little pillars of plaster-of-Paris, the lower ends of which were immersed in water, emptied themselves in thirteen days. Precautions were taken to exclude the action of bacteria. Puriewitsch found that the plaster pillars were unnecessary, and that the same object could be effected by keeping the ends of the endosperms in contact with sufficient water. Too little water or the presence of an anæsthetic arrested the action.

The experiments of H. T. Brown and F. Escombe¹ proved the accuracy of Pfeffer and Hanstein's observation that the endosperm is not wholly passive during germination, but takes some share with the embryo in preparing the reserve materials for the use of the plantlet. Brown and Escombe point out however that the changes which take place in the endosperm when separated from the embryo and placed under favourable conditions are to be ascribed to the aleurone layer alone (which can, in fact, withstand exceedingly drastic treatment without being killed), since fragments of the endosperm deprived of their aleurone layer when steeped exhibited no visible changes until micro-organisms appeared.

Action of the Proteolytic Enzymes.—At the time that the carbohydrate portions of the endosperm are being broken down there can be no doubt that a similar action takes place in the proteids, evidently through the instrumentality of one or more proteolytic enzymes, but we are still in ignorance as to where or how these originate. The proteid grains of the cells of the scutellum disappear at a very early stage in germination; and, subsequently, solution and absorption of the entire contents of the endosperm, which include a considerable amount of proteid matter, takes place. Hence it is only fair to presume that a solvent action analogous to that which occurs with the carbohydrates is at work. We know that much of the insoluble proteid matter of the barleycorn is converted during the malting process into a soluble condition, and that a large proportion of it is also broken down to the amide form, in which state it is not only soluble but eminently diffusible.

The proteid grains of the aleurone layer are the last to be affected; no trace of solvent action can be detected in them until the acrospire projects some one-fourth or one-fifth of an inch beyond the end of the barleycorn; consequently, they are only appropriated at a late stage of germination.

¹ *Proc. Royal Soc.* 1898.

These cells apparently undergo no change during the limited time which the germination is allowed to proceed in malting.

Since the barleycorn, during all those stages of its growth which concern the maltster, derives the energy expended in the building up of its tissues from the combustion of carbon, the seed loses weight, and as the loss of weight falls principally on the starch, it is evident that all unnecessary growth implies a waste of starchy material. The barley germ, during that period of its growth in which it is unable to manufacture its own carbohydrate food from the carbon dioxide of the atmosphere, is indebted to the green leaves of the plant on which it was formed for its supply of this nutriment, since these were the agents concerned in the manufacture of the starch stored in its endosperm.

CHAPTER V

FERMENTATION

EVEN at the present time it is somewhat difficult to give a clear definition of the term fermentation; J. Reynolds Green¹ defines it as the decomposition of complex organic material into substances of simpler composition by the agency either of protoplasm itself or of a secretion prepared by it. It will be observed that this definition includes the reactions brought about by those agents known as enzymes which have been considered in an earlier part of this work. Indeed it now appears probable that all fermentation phenomena are the result of enzymes some of which are so closely related to protoplasm that they cannot be isolated.

Antiquity of Fermentation.—The process of fermentation, with its accompanying phenomena, was evidently known at a period long antecedent to that of which we have any historical record, for the earliest known writings distinctly show that, at the time they were composed, not only was the comparatively simple method by which wine is produced from the grape in full operation, but that the more complicated processes by which fermented liquors are made from barley and other cereals were also in common use. The ancients knew, also, how to manufacture vinegar, a substance produced by submitting an alcoholic liquid to a subsequent fermentation of an entirely different nature. The practical application of fermentation to bread-making also dates from a very early period.

Beer, and the employment of barley in its manufacture, are frequently mentioned in the ancient Egyptian *papyri*. For instance, the Westcar Papyrus,² now in the Berlin Museum, contains frequent allusions to beer and barley being given as presents to the living or as offerings to the dead. This papyrus was most probably written in the twelfth dynasty, and the tales it contains were presumably folklore at a period long antecedent to this; without doubt, they date back to several thousands of years before the commencement of the present era. It is impossible to say what was the nature of the beverage brewed from barley in these days, but that it was a liquid, and not a semi-solid article, as has been suggested, is shown by a remark which occurs in the Ebers Papyrus, where directions are given to infuse a certain quantity of a particular herb in a certain quantity of beer.

¹ "The Soluble Ferments and Fermentation," Cambridge, 1899.

² For an exceedingly interesting translation and account of this papyrus see "Egyptian Tales" (First Series), by W. M. Flinders Petrie.

Although the ancients possessed an extensive practical knowledge of fermentation, and though we cannot but feel certain that many an intelligent individual, even in those primeval times, must have been struck with its singular and almost mysterious attendant phenomena, and have been induced to reflect on the subject, yet only in comparatively recent times do we find attempts made to give a theoretical explanation of the process. One of the first known instances of this occurs in Pliny's "Natural History," in which a definite statement is made that the fermentation of dough in bread-making is effected through the instrumentality of an acid body. As history progresses, however, we meet with frequent references to the theoretical aspect of the subject, in which opinions of the strangest and most varied nature are propounded from time to time, and which were materially influenced by the prevailing phase of chemical theory of the period in which they were enunciated.

Views of Fermentation during the Period of the Alchemists (about from 350 to 1525 A.D.).—After the fall of the Roman power the seat of science became transferred to Arabia. During the earlier portion of the period which we have under discussion, the Arabians devoted their scientific energies chiefly to observing and recording natural phenomena, as well as to making experiments, and even now we must regard with astonishment the vast and varied amount of knowledge accumulated by this people at that remote period of history. Already in the eighth century Geber taught the method of preparing vinegar, and in the eleventh century Alzaharavius knew how to separate spirit from a fermented liquor by distillation. Traces of this knowledge are still met with in our common orthography; many of those words which begin with *al*, such as alcohol, algebra, alchemy, &c., are derived from the Arabic, and were expressions evolved during the period referred to.

This period of observation and experiment was succeeded by that of alchemy pure and simple, and though it was an age of scientific error, still it was one in which much experimentation took place, and this led to the compilation of an enormous mass of chemical facts, as well as to the discovery of many new bodies. As astrology was the precursor of astronomy, so may alchemy be regarded as the foundation upon which the science of chemistry was subsequently built. The alchemists fondly imagined that it was possible to find a substance, vaguely termed "The Philosopher's Stone," which would be able to turn the baser metals into gold, to heal every disease, and confer omniscience on its possessor. Led by the pursuit of this chimera, thousands of the best minds in Europe expended their skill and energy for centuries, and performed countless numbers of experiments for the furtherance of this vain object. In the writings of this period we often meet with the expression *fermentatio* but it seems used in a somewhat vague way, and appears to be frequently employed indiscriminately with the words *digestio* and *putrefactio*. Any chemical reaction which was accompanied by external phenomena of a marked character such as effervescence, was called a fermentation. The action which ensues when an acid is poured over chalk was called a

fermentation, since the action was accompanied by the copious evolution of gas. No distinction, however, was made between the reactions which took place between inorganic bodies and those which occurred between organic substances.

The word "ferment" is also frequently met with, and seems to have been similarly used in a vague and dubious sense. Most frequently it appears to imply a substance capable of exciting some kind of germination, and generally seems intended to denote a body endowed with the power of causing a metamorphosis (*transmutatio*) in other bodies. The "Philosopher's Stone" was even looked upon as a kind of ferment, which was to set up a fermentation in the commoner metals, such as lead or quicksilver, and, as it were, awaken in them that dormant germ which, when aroused, should, by a species of germination, ennoble those metals, and finally convert them into the noblest of metals—gold. The view that the process of fermentation had a purifying and elevating effect on the bodies which had been submitted to its influence seems to have been held not only by the alchemists, but also by the philosophers of a later age. Thus Basil Valentine, in the fifteenth century, considered that when yeast was added to wort an internal inflammation was communicated to the liquid, which determined a perfection and separation of the clear parts from the turbid. The fact that alcohol was present in a fermented wort was well known to him, but he imagined it to have been pre-existent in the decoction of germinated barley, and that by fermentation it was freed, as it were by a refiner's fire, from all the contamination and impurities which accompanied it, and which had hitherto prevented it from exhibiting its true properties. This was supposed to be the reason why alcohol could not be obtained by distilling the wort before it had undergone fermentation. It was held back by its accompanying impurities; when freed from these, it could be obtained by distillation.

Basil Valentine may be regarded as the last of the alchemists, for, at the time he lived, knowledge of the facts of nature had progressed to such an extent that philosophers could not help perceiving the utter futility of their search after the "Philosopher's Stone," and when this came to be fully recognised, they began to turn their efforts in other directions, where there seemed to be a greater probability of their being rewarded with substantial results.

The Period of Iatro-Chemistry.—During this period, which immediately succeeded that of the alchemists, science was regarded as being merely subservient to medicine, and the investigations which were undertaken during this time kept this particular idea in view. Fermentation seems to have largely occupied the attention of scientists, and we find many new hypotheses proposed for its explanation.

Libavius (1595) no longer regarded digestion and fermentation as the same, but as allied processes. He was of opinion that the ferment must have a close natural relation to the fermentable body; he also showed

that a body will not ferment unless it is in a state of solution or of fine division; he also noted the effect of temperature on fermentation.

In 1648 Van Helmont published several works on fermentation. His views on the subject were of the most remarkable nature. He considered that not only were all such purely chemical phenomena, as the effervescence which ensues when an acid is poured on an alkaline carbonate, processes of fermentation, but that every physiological action carried on in the animal economy, such as the formation of intestinal gases, the formation of the blood itself, &c., were similar processes, and even that small animals could be formed by its agency.¹ Amidst these extravagant views we find others of a more reasonable nature, as, for instance, that in which he states that out of the ferment something passes into the fermenting liquid which grows in it as a seed vegetates in the earth, and through its agency the fermentation is brought about. This is to a certain extent in agreement with modern views of the subject, and had Helmont discovered the fact that it was the ferment itself which grew, and thereby caused fermentation, he would have anticipated the modern view of Pasteur. Helmont also recognised the fact that the escaping gas was an entirely different substance from the alcohol left behind; and that this gas, which he calls "gas vinorum," was derived from something which, without fermentation, would have appeared as carbon.

Sylvius de la Boë, in 1659, distinctly perceived the marked difference in the nature of the two processes, fermentation and the effervescence which occurs on the addition of an alkaline carbonate to an acid. He says that though an evolution of gas occurs in both instances, in the former case the compound acted upon suffers decomposition; in the latter, a new compound is formed.

The views expressed on the subject of fermentation in the writings of Becker show a marked advance on those of his predecessors. He points out that fermentation can only be induced in sweet liquids—that the alcohol is not pre-existent in the fermentable fluid, but is formed during the fermentation. He regards fermentation and combustion as analogous processes, a view which is consistent with present knowledge. He seems also to have had an indistinct idea that the presence of air was necessary to fermentation. According to his views, there are three distinct kinds of fermentation; the first that which we call now effervescence, the second the alcoholic fermentation, and the third the acetic fermentation. He looks upon fermentation and putrefaction as similar but distinct processes, and distinguishes them by the character of their products; in the first case the substance is improved in its

¹ Helmont believed that the effluvia arising from morasses produced frogs, snails, leeches, &c. But still more curious is the receipt which he seriously gives for producing a pot of live mice. To effect this, he says, it is only necessary to close the mouth of a pot containing a little corn with a dirty shirt. The ferment evolved from the shirt, modified by the odour of the corn, effects in about three weeks time the transformation of the corn into live mice. These, according to Helmont, who states that he has actually witnessed the phenomenon, are born full grown, and of both sexes, and that, in order to continue the progeny, it is only necessary to pair them.

nature, in the second it is deteriorated. He made the important observation that the juice of grapes, the skins of which had not been ruptured, never entered into fermentation, and this he considered to be due to the fact that the juice never came into actual contact with air. He recognised the fact that alcohol and salt of tartar, when present in sufficient quantity, impeded and finally arrested fermentation.

Willis, an English physician, who wrote in 1659, attributed much importance to fermentation: he considered that all the processes carried on in living bodies are caused by its agency, and that diseases are caused by fermentations which have deviated from their ordinary healthy course. He regards a ferment as a body which is able to communicate a portion of its own internal movement to another body, and thus to induce in it fermentation. This view of the subject, which is known as "the mechanical theory of fermentation," afterwards, with certain elaborations, formed the basis of the theories of Stahl and Liebig.

Views on Fermentation during the Age of the Phlogiston Theory.—With the enunciation of the theory of phlogiston by Stahl in 1697 there commenced a new era in philosophy. The chief merit of Stahl and his school was that they no longer cultivated science as a subject dependent on some object, but for its own sake alone. Natural phenomena were no longer investigated in the light of their utility or otherwise to mankind, but simply in order that new truths might be established.

The doctrine of phlogiston, which was propounded by Stahl to explain the phenomena of combustion, dominated the scientific world for a very long period. According to this theory, every combustible substance was a compound of phlogiston and some other body. The phenomena of combustion in a body were caused by the escape of the phlogiston which it contained, and the more of this hypothetical substance entered into its composition the more combustible was that body. Such a highly combustible body as alcohol was supposed to contain a large amount of phlogiston. The adherents of the theory were well aware of the fact that the weight of a dephlogisticated body was often greater than its original weight, and they sought to explain this anomaly by assuming that phlogiston was a body possessing negative gravity, and that, though every other body in nature was attracted to the earth by the force of gravitation, it was repelled. Consequently, phlogiston, when present in a substance, tended to buoy up and render it lighter; hence, after its escape, the substance became heavier.

Stahl paid much attention to the subject of fermentation, and recorded his peculiar views on it in a work, *Zymotechnica Fundamentalis*, which was, however, only published after his death. He regarded fermentation and putrefaction as analogous processes, and considered that a body which is itself in a state of putrefaction can readily cause another body, capable of undergoing a similar change, to assume a like condition; such a body, itself in a state of active internal motion,¹ can readily communicate this motion to

¹ This expression "internal motion of the particles of a body," and the previous one

another body, which, though its particles may be at present in a state of rest, are easily set in motion. Stahl sought to explain the reason why bodies are produced during fermentation which differ from those originally present in the liquid by assuming that the ultimate particles¹ of which the original substances were composed (salt, acid, combustible substance, water) are first disassociated by the internal motion excited by the ferment, these then recombine to form more stable compounds, in which the same elements exist, but are combined in different proportions. If fermentation took such a direction that the combustible particles predominated in the newly-formed body, then it was alcoholic; if acid particles predominated, then it was an acid fermentation. For more than eighty years the views of Stahl were held by the members of the scientific world, amongst whom we find some of the most honoured names; and though modifications were proposed from time to time by his immediate successors, they were of an unessential nature. One of these, enunciated by Boerhave, a celebrated scientist and physician, was, that substances of animal origin alone were capable of undergoing putrefaction, whilst those derived from the vegetable kingdom were alone capable of entering into fermentation. A German chemist, Wiegleb, endeavoured to show that the products of fermentation were already present in the liquid before fermentation, but this was merely a return to the older doctrine. An attempt was made to obtain a more intimate knowledge of the nature of fermentation by a study of the gases which were evolved during the process. The English chemist Cavendish, in 1766, not only established the identity of the gas evolved during fermentation with that evolved when an acid is added to an alkaline carbonate, but also actually measured the gas. He found that when 100 parts of sugar were fermented, 57 parts of carbon dioxide were evolved; and this may be looked upon as the first experiment in which an attempt was made to quantitatively estimate the products of fermentation.

Views on Fermentation during the Period of the Foundation of Modern Chemistry.—The distinguished French chemist, Lavoisier, who was born in 1743 and died in 1794, may be regarded as the founder of modern chemistry; he and his associates effected a complete reform in chemical philosophy, and in this way effectually banished the theory of phlogiston. The doctrines of Stahl and his school, both on this subject and on that of fermentation, then gradually fell into oblivion. By the extensive application to his work of a systematic method of measuring and weighing, as accurate as the means at his command would permit, Lavoisier introduced methods of precision into the domain of chemistry which had been hitherto unknown. He determined the relative proportion of the elements in water and in the atmosphere; discovered the important part played by oxygen (which element had of Willis, must not be confounded with that modern view of the vibration of the ultimate atoms of matter, as we now understand it, when speaking of the mechanical theory of heat.

¹ It must be remembered that at this period the elements, as we regard them at the present day, were unknown.

been previously discovered by Priestley in 1774) in the process of combustion, and this had a profound influence on the views then held with regard to the phenomena of fermentation; he also showed that oxygen was absolutely necessary for the acetic fermentation. He paid considerable attention to the subject of fermentation, and estimated quantitatively the elementary composition of sugar and alcohol. By weighing the sugar introduced into a liquid previous to fermentation, and estimating the respective quantities of the alcohol and carbon dioxide formed during that process, he found that the weights of the two latter bodies when added together were equal to that of the original sugar, and hence came to the conclusion that, in fermentation, the sugar was simply converted into alcohol and carbon dioxide. Though the figures he obtained are now known to be far from accurate, this cannot be wondered at when we consider the primitive nature of the apparatus at his command, and it was only by a singular series of compensating errors that he obtained this result. He believed that the yeast suffered no chemical change in the process, for, as the whole reaction was explained quantitatively in the above manner, he did not see any place for the participation of the yeast. In his first formula, published in 1789, Lavoisier does not seem to have regarded the formation of acetic acid, which body he invariably found to be produced in all his fermentations, as an essential part of the process; afterwards, however, he came to a different conclusion, and propounded formulæ in which the formation of acetic acid also was accounted for. Fermentation from his point of view being purely a chemical process, he regarded sugar as an oxide, which during fermentation was split up into two other oxides; the one (carbon dioxide) richer in oxygen, the other (alcohol) poorer in this element.

The next important theory on this subject was advanced by Fabroni in 1787, and this excited great attention at the time it was propounded. Fabroni, who regarded fermentation as a purely chemical process, considered that yeast was very much allied in nature to the gluten of plants, and he demonstrated by experiment that fermentation could be induced in a saccharine liquor by the addition of gluten. He also proved experimentally that fermentation could not be induced in uninjured fruits, and drew from this the correct conclusion, that, in order that fermentation may be induced, the ferment and the fermentable body must be in actual contact. He imagined that the carbon dioxide gas evolved during fermentation was evolved from the ferment, and not from the fermenting substance; also that the alcohol was not pre-existent in the fermented fluid, but only arose during the distillation.

Although many chemists continued to turn their attention to the phenomena of fermentation, nothing of a particularly important nature was contributed to the subject until we come to Gay-Lussac, who propounded a fresh theory. In this he considered that oxygen was the sole cause of all fermentation phenomena, be they those of the alcoholic fermentation, of putrefaction, or of decay. According to his view, fermentation was caused by a body

which, though not itself possessed of as much stability as the fermentable matter present in the solution, was acted upon by oxygen, under the influence of which its particles were set in motion. This motion being communicated to the particles of the other and more stable body, they were likewise set in motion, and thus the whole body was brought into a state of fermentation. Gay-Lussac performed many experiments which seemed to confirm his conclusions. In one of the most important of these he enclosed some uninjured grapes (after having washed them in an atmosphere of hydrogen) in a vessel, which was then filled with air-free mercury. The grapes were then pressed in such a way that the grape-juice did not come in contact with air. The juice obtained under these circumstances did not enter into a state of fermentation, whereas it was notorious that when grapes were expressed in the usual manner in the presence of air the juice speedily fell into a state of active fermentation. When, however, the smallest quantity of pure oxygen was admitted to the grape-juice floating on the surface of the quicksilver in the former case, fermentation speedily commenced. The well-known action of sulphurous acid in arresting fermentation also lent a powerful support to this theory. This acid has a powerful affinity for oxygen, and its marked effect on the process of fermentation was attributed to the complete manner in which it removed the oxygen from the fermenting liquid. A method for preserving provisions had been introduced to public notice by Appert about this time (1810), which apparently added considerable weight to Gay-Lussac's view. By this process provisions of the most varied nature, such as soups, meat, vegetables, &c., were preserved for an indefinite period by enclosing them in a perfectly air-tight vessel, which was subsequently exposed for a certain length of time to the temperature of boiling water.¹ The method is very similar to the one of which such frequent use is made at the present day, and by means of which much valuable food is imported from distant countries.

It was sought to explain the rationale of the process by assuming that the small quantity of oxygen present after the vessel had been sealed up combined with the substance contained therein, and being in this way withdrawn from the sphere of action, was not permitted to exercise its power of inducing fermentation. Another curious fact had been observed which seemed to add considerable support to Gay-Lussac's theory. A bottle of grape-juice which had been preserved for a year by Appert's process was uncorked and transferred to another bottle, when it soon fell into a state of fermentation. It was supposed that the fermentation was caused by the short contact of the liquid with the oxygen of the atmosphere which took place during its transference from one vessel to the other.

Thénard, to whom we owe some important contributions to the science of fermentation, framed the following theory on the subject, in which oxygen played an important part. He assumed that the yeast withdrew oxygen from

¹ An exactly similar process for the preservation of vinegar had been proposed by the celebrated Swedish chemist Scheele in 1782.

the sugar, and in this way effected its conversion into alcohol and carbon dioxide. He also demonstrated the incorrectness of the theory of Fabroni, by showing that purified gluten was unable to excite alcoholic fermentation in a saccharine liquid.

Meanwhile, from the time of Lavoisier to that of Gay-Lussac, chemical apparatus had been considerably improved, and chemists were enabled to obtain much more accurate analyses of many of the commoner organic substances met with; amongst these were alcohol and sugar. It was also shown that alcohol was present in the fermented liquid before distillation, a matter on which some uncertainty had previously prevailed. After studying the composition of the substances taking part in the process of fermentation, in the light of the more correct knowledge of their actual composition, and drawing his deductions from a consideration of the decomposition which ought to occur rather than by direct experiment, Gay-Lussac was led to propose the following equation in explanation of the change which takes place when sugar is subjected to the alcoholic fermentation:—

This formula is found in the text-books of the present day, and is so near the truth that it is the one we now invariably employ when we require a short and concise expression for the reaction which takes place during the alcoholic fermentation of sugar.

Between the time of Gay-Lussac and Liebig, fermentation continued to engage the attention of many men of science, and various views were propounded by Trommsdorf, Fourcroy, and others. Amongst these, Kamtz expressed the peculiar view that fermentation was an electrical phenomenon, that sugar formed the negative and the yeast the positive element in an electric couple, and, under the influence of the electric tension, the sugar was split up into the negatively-electric carbon dioxide and the positively-electric alcohol. Berzelius invented the name "catalysis" to explain a number of phenomena which it was not possible to account for at that time by the ordinary laws of chemistry. The characteristic of such a body is the power which a small quantity possesses, without itself undergoing any change, of inducing a change in a large quantity of another substance. He sought to explain the process of alcoholic fermentation by assuming that yeast was a substance endowed with an immense catalytic power, in virtue of which it was able to set up decomposition in large quantities of a fermentable body. With the progress of time many of the processes which were referred to catalysis by Berzelius have found their true explanation.

The Views of Liebig on Fermentation.—We now come to the theory of Liebig, propounded in 1839, that ruled the scientific world for thirty years, and which has undoubtedly much in common with that proposed by Stahl a hundred years before. In both these theories the ferment is regarded as a body which is itself in a state of decomposition, and which is able to communicate this peculiar state to other bodies of a more stable nature; it is,

in fact, a sort of infection. Both theorists assume the hypothesis that the smallest particles of a decomposing body are in a state of movement, and that when such a body exists in solution along with another body, also to some extent of an unstable nature, the former is able to communicate this motion to the latter. Bersch,¹ in illustrating the action which, according to this theory, takes place during fermentation, mentally pictures a single snowflake which commences to roll down a mountain-slope covered with snow. In rolling, it carries along with it another snowflake, and these in turn communicate motion to others, until at last the flakes set in motion are so numerous that an avalanche is the result, which, from the collective energy of the enormous number of particles of which it is made up, is capable of exhibiting the mightiest effects of force. So, in like manner, the decomposition actually taking place in the smallest trace of a ferment may be transferred to, and occasion the decomposition of, almost unlimited quantities of a fermentable substance. This view also yielded a rational explanation of the well-known fact that an almost imperceptible trace of yeast is able to ferment away an infinitely large amount of fermentable matter.

But whilst Stahl only proposed this action as an explanation of the phenomena of fermentation, Liebig endeavoured to show that it was merely one of the ordinary manifestations of the chemical laws common to the inorganic as well as the organic realms of chemistry, and he brought forward innumerable examples in attestation of this view; such, for instance, as the solution of platinum when alloyed with silver in nitric acid; the action of hydrogen peroxide in solution, which, when brought into contact with many metallic oxides, not only gives off its own oxygen, but also induces the metallic oxides, to yield up a portion of their own oxygen; the action of nitric oxide in the manufacture of sulphuric acid, &c. He also adduced the action of enzymes, which at that time were all classed together under the category of ferments, and considered that when a true explanation of the action of these should be found, it would also elucidate the action of the organised ferments. Bodies of an explosive nature were also adduced; these have their particles in such a loose state of combination, that moderate heating or a slight blow causes their separation. He held that, to a certain extent, the fermentable bodies were similarly constituted, their particles only being held together by the "force of inertia," or, as we should now say, by a weak chemical affinity. The slight impulse given to such compounds by the ferments sufficed to upset their equilibrium, and to induce in them a decomposition of a nature somewhat akin to an explosion.

Certain observations which had been made before Liebig's time lent an apparent confirmation to this view of the nature of ferments. Thénard had shown that yeast, when left to itself in a moist condition, rapidly fell into a state of putrefaction; Colin had found that yeast diminished in quantity during fermentation; Döbereiner had discovered that ammonia was produced during fermentation, and the formation of this substance is an almost invariable

¹ *Die Hefe u. d. Gährungs-Erscheinungen*, i. 28.

concomitant of the putrefaction of nitrogenous organic matter. In the two latter cases the facts observed were only true under certain conditions, and they never occur when a fermentation pursues a normal course. Liebig considered that fermentation, putrefaction, and decay were all caused in the same manner; fermentation was only a special case of putrefaction, in which the oxygen of the atmosphere co-operated, and in which the fermentable body was a nitrogen-free substance, just as the formation of acetic acid in the manufacture of vinegar might be regarded as a putrefaction of alcohol.

The fact that yeast was a living organism was to Liebig a matter of indifference; he considered it merely an accidental circumstance, and that its growth and other vital phenomena were in no way concerned with the process of fermentation. He adduced in support of this argument the production of acetic acid from alcohol, which could readily be effected without the intervention of a living organism.

In later times, when the views of Pasteur began to meet with some acceptance in Germany, Liebig brought forward the following objections to them in a lecture delivered before the Bavarian Academy in 1868. He contended that, as all yeast contained a large amount of proteid matter, of which sulphur is an essential constituent, he could not conceive that, as had been asserted by Pasteur, yeast multiplication could take place in a solution consisting solely of water, sugar, ammonia, and "phosphates." With the increase of yeast new proteid matter must be formed, and this was impossible in the entire absence of sulphur. Pasteur had, however, when he spoke of "phosphates," meant the ash of yeast, and this contains a notable amount of sulphates. Objection was also taken to the reason assigned by Pasteur for the disappearance of the ammonia, who regarded it as being utilised in the formation of new proteid substance; Liebig affirmed that it became converted into ammonio-magnesium phosphate, and as such was precipitated and withdrawn from observation. It has, however, been shown since then that yeast can obtain its supply of nitrogenous food from this salt.

This was followed, in 1870, by a series of articles in the *Annalen der Chemie und Pharmacie*, in which Liebig reiterated his former objections, and made a strenuous effort to overturn the theory propounded by Pasteur. At the same time considerable concessions were made; Liebig not only no longer denied that yeast development was a necessary concomitant of fermentation, but also completely renounced his previous theory of 1839. Still he attempted to explain the process in a way which should to some extent resemble his previous theory. In this he assumed that, during the life of the yeast, there was formed in the interior of the yeast-cell an enzyme, similar to diastase or invertase, which had the power of converting sugar into alcohol and carbon dioxide; and that the only correlation between the physiological act and the phenomena of fermentation is the production in the living cell of a substance which, acting as an enzyme, effects the decomposition of the sugar. The physiological act is only necessary for the production of this substance, and in no other way is it concerned with

fermentation.¹ This formed Liebig's last contribution to the subject of fermentation.² He died in 1873. The enzyme theory of alcoholic fermentation has now been completely proved by E. Buchner (*see* p. 355).

Traube's Views on Fermentation.—A new theory of fermentation was enunciated by Traube in 1858. According to this, an enzymic body, having a definite chemical composition, being closely allied in its nature to the proteïds, and having also a powerful affinity for oxygen, is formed in the interior of each fermentation organism. This he considered to be endowed with the peculiar property of taking up oxygen from one portion of the molecular group of a fermentable body and transferring it to another portion, but that it did not act in the manner suggested by previous observers, *i.e.*, by transferring the motion of their own particles to those of other bodies. He divided these supposed enzymes or oxygen carriers into two groups, the first of which absorbed free oxygen and transferred it to another body, as in the conversion of alcohol into acetic acid; consequently, for the performance of their special function they required the presence of free oxygen. The second group, which he called "reducing enzymes," were supposed to transfer combined oxygen, and to this class belong the alcoholic ferments. In order to explain the action of the latter, he assumed that fermentable bodies consisted of two molecular groups, the former of which had an affinity for hydrogen, and as the enzyme had an affinity for oxygen, the two, acting conjointly, were able to tear asunder a molecule of water. The hydrogen of the water passed to this first molecular group, and the oxygen to the enzyme, and this constituted a true reducing action. The first group was the one which became alcohol in the alcoholic fermentation. The second molecular group possessed an affinity for oxygen, and this was satisfied by the oxygen that the enzyme had torn from the molecule of water, and which it now yielded up to this group; this in the alcoholic fermentation became carbon dioxide. During the course of this action the enzyme was not destroyed, but simply acted as an intermediary agent. As the oxygen, according to this theory, was entirely derived from water, this particular class of fermentation was able to proceed in the entire absence of free oxygen.

The Physiological or Vital Theory of Fermentation.—During the period we have just been discussing, the view that fermentation was intimately connected with the growth and development of the yeast organism had steadily been gaining ground amongst investigators; but owing in great measure to the vast authority of Liebig at that time it was discredited, and even ridiculed.³

¹ To this view Pasteur raised no objections (*vide Études sur la bière*, p. 315).

² *Theorie der Fermentwirkungen*, Berlin.

³ A curious specimen of this form of sarcastic ridicule, which undoubtedly came from the incisive pen of Liebig, was published in the *Annalen der Pharmacie* (vol. xxix, p. 100), one of the first and most serious scientific periodicals of that day. The writer professed that he had examined beer-yeast under the microscope, and found it to consist of an infinite number of minute spheres, which, when placed in a solution of sugar, were found

Yeast was examined for the first time microscopically, and its organised structure recognised, by Leeuwenhoek, in 1680. He found that it consisted of spherical bodies, which he was, however, unable to distinguish from starch-cells; but we cannot wonder at this when the rudimentary form of the first microscopes is taken into account. Thénard, at the beginning of the present century, expressed the view that yeast was of an animal nature, but he only arrived at this conclusion on purely chemical grounds; he found it was extremely rich in nitrogen, and resembled in this respect the composition of animal bodies. In 1818 Erxleben, after examining yeast under the microscope, definitely pronounced an opinion that it was of a vegetable nature, and that it must be regarded as the cause of fermentation. In 1822 Persoon microscopically investigated the film which occasionally appears on wine, and found it to consist of cells very similar to yeast-cells. Desmazières, in 1825, confirmed this observation, and considered the two sets of cells to be very nearly allied organisms; he, however, believed them to be of an animal nature. About this time it began to be generally conceded that yeast consisted of vegetable organisms. Cagniard de Latour, in 1835, made an exhaustive examination of yeast under the microscope, and found it to

FIG. 55.—Yeast Growing in a Fermenting Fluid.

consist of minute spherical organisms, which reproduced themselves by means of buds. The appearance of yeast growing in a fermentable liquid is given in the accompanying figure (Fig. 55); in this, yeast-cells in various stages of growth and development may be seen.

As these organisms did not exhibit any power of movement, Latour considered that they were of a vegetable nature. He did not, however, completely abandon the old ideas, but held that in some cases oxygen might be

the cause of fermentation. In 1838 Turpin formulated the vital theory of fermentation. He said: "Fermentation as effect, and vegetation as cause, are two things inseparable in the decomposition of sugar." About the same time Kützing and Mitscherlich, independently of one another and other observers, came to the conclusion that beer-yeast consisted of organisms which were intimately connected with fermentation, though the latter considered to be eggs, for they rapidly developed into little animals having the appearance of an alembic without a receiver. That which resembled the tube of the helmet was a kind of trunk covered with long hairs. Though no teeth or eyes could be seen, a stomach, digestive canal, and urinary system were distinctly visible. These exceedingly voracious beasts greedily devoured the sugar, which, after undergoing digestion in the stomach, was excreted partly as alcohol from the digestive tract, and partly as carbonic acid by the urinary system. Such was the voracity of these little creatures, that they devoured and excreted in eighteen hours sixty-six times their own weight of sugar. When all the sugar had been eaten up the animals perished, leaving behind them a plentiful supply of eggs, and these constituted yeast.

that the organisms only acted by catalysis. It had now been decided by the majority of investigators that yeast was a vegetable organism, and the question arose as to what position should be assigned to it in that kingdom. To some it appeared to be a fungus without mycelium, to others it closely resembled the *Algæ*. Whilst Turpin regarded the organisms as the spores of plants belonging to the genus *Torula* of Persoon, Meyen considered that yeast was a fungus, and created for it a new genus, the *Saccharomyces*; this name was also adopted by Reess, Engel, &c. Kützing, who regarded the ferments as *Algæ*, arranged them in a separate genus of that class, which he named the *Cryptococci*. Later on we shall see that the yeast organism is capable of reproducing itself by spores, and now it is generally conceded that it belongs to the fungi.

We now come to the important work of Schwann, carried out during 1836-37, for with it commenced the real solution of the question. Though undoubtedly he arrived at his conclusions at a period subsequent to that of Erxleben, Latour, &c., yet it must be placed to his credit that he not only stated the proposition that fermentation was due to the presence of living organisms, but he also proved the correctness of his views by a number of the most ingenious experiments. Many years after the publication of the experiments of Schwann, we see them repeated in almost the same way, and with the same results, by Pasteur. It was, however, reserved for this latter investigator to obtain general acceptance for the vital theory of fermentation.

The Doctrine of Spontaneous Evolution.—At the time Schwann carried out his investigations, this doctrine, which assumed that it was possible for living organisms to be developed out of lifeless matter without the intermediate agency of eggs, seeds, or germs, was engaging the serious attention of the scientific world, which it divided into two great parties, the one affirming that living organisms could originate from lifeless matter, the other that this never occurred. It was with a view to solving this problem that the experiments and observations of Schwann were undertaken. The well-known fact that an animal or vegetable infusion, when left to itself for a time, was invariably found to teem with living organisms, seemed to give some weight to the opinions of the upholders of the theory of spontaneous evolution; and as these organisms were of the simplest nature, an investigation into their origin and development seemed to be peculiarly adapted for the solution of the problem.

Some very curious opinions on this subject had been held from ancient times to the end of the Middle Ages; it was then an accepted fact that even large organised beings, such as mice, frogs, snakes, caterpillars, &c., were evolved spontaneously. These errors had been to a certain extent exploded by the criticism of the seventeenth century; but when, during the latter part of that century and the earlier part of the eighteenth, the microscope came to be largely employed, and by its means there was discovered a host of minute organisms in connection with which no sexual reproduction could be observed, then again the theory of spontaneous evolution became a prominent question.

It was assumed that particles of matter, which at one time had entered into the composition of organised beings, after the death of these possessed the power, under favourable circumstances, of uniting into new forms of animated beings. On the other hand, there were always philosophers who maintained that this was not the case. The question of spontaneous evolution was brought prominently into notice by Needham, an English monk, who wrote in 1745, and also by Buffon in 1749. They showed that an aqueous extract of meat placed in closed vessels, after exposure to the heat of boiling water, was able to develop living organisms, which these observers conceived could have been formed in no other way than by spontaneous generation. Many other observers took an entirely opposite view on the subject, one of the most notable of whom was Spallanzani; he commenced in the year 1765 the publication of the results of a series of experiments, the results of which were in direct contradiction with those of Needham. Spallanzani exposed hermetically sealed flasks containing meat-extract solution for an hour to the temperature of boiling water, and found that in these no organisms made their appearance afterwards. Immediately, however, the seal was destroyed, and atmospheric air allowed to have access to the contents of the flasks, organisms quickly developed. From this he concluded the organisms did not arise spontaneously, but were derived from germs present in the air. In the former case these had been destroyed by the heating, but in the latter they obtained access to the flask in an unaltered condition, and consequently were able to develop. To these experiments Needham and his adherents made the following objection: that, during the heating, the contents of the flasks had been so maltreated that they had lost their power of vegetative reproduction, and that the small quantity of air included in the flask had also been similarly corrupted.¹ Schwann now proved that air might be admitted in any quantity to solutions which had been boiled in flasks, without causing fermentation or putrefaction, provided the germs contained in such air were destroyed. This was effected in the following manner: Each flask was provided with a stopper perforated with two apertures, into which tubes bent at an angle were inserted; one of these served for the introduction of air which had been previously passed through a red-hot tube. In this way he showed that though large quantities of air might in this manner be passed through the liquids in the flasks, in no case did fermentation or putrefaction take place in their contents. About the same time Schulze performed a series of experiments with flasks of a similar construction, but instead of heating the air, he passed it through strong sulphuric acid; he obtained results similar to those of Schwann. It was now objected by the opposite party that in all these experiments the air had been so altered in the treatment it was subjected to before being passed through the solution that it had lost its power of causing the dead particles to reform themselves into living organisms. To demonstrate that this was not the case, a series of ingeniously

¹ It must be remembered that at this time nothing was known definitely of the chemical constitution of the atmosphere.

contrived experiments were made in 1854 by Schröder and Dusch, in which the air, instead of being either heated or passed through strong acid, was simply caused to pass through a filter of cotton-wool.¹ Results similar to those of Schwann were obtained by this modification in all cases, with the exception of milk, so that even yet there was something that remained to be explained. It was, however, distinctly proved that such a liquid as beer-wort or grape-juice could be rendered completely sterile by boiling, and that by means of a simple filter of cotton-wool it was possible to remove those organisms from the air which caused fermentation or putrefaction. Undoubtedly the flasks of Schwann were the precursors of those so much employed afterwards by Pasteur and other investigators; and the cotton-wool filter invented by Schröder and Dusch is largely employed at the present time, both in the laboratory and in the apparatus for the cultivation of pure yeast.

Pasteur's Investigations on the Question of Spontaneous Generation.—In the pursuit of his studies on fermentation Pasteur was compelled to inquire into this subject, and this he did in such a masterly manner that many felt convinced that the question had been settled for ever.

He had previously observed that when solutions of sugar, to which small quantities of the magnesium and potassium phosphates and a little ammonium sulphate had been added, were kept warm for a time, fermentation, accompanied by the growth and development of certain bacteria, almost invariably set in, during which the sugar gradually became converted into lactic acid. When this stage had been reached, the mixture, if allowed to remain for some time longer, entered into another fermentation, accompanied by an entirely different set of bacteria, during which the lactic acid gradually became transformed into butyric acid. As such a solution, in which nothing but mineral substances were present, seemed peculiarly unfitted for the spontaneous generation of living organisms, Pasteur was led to conclude that the germs of these two different organisms could have obtained access to the liquid in no other way than by falling into it from the atmosphere. If this were the case, then it ought to be possible to find these organisms in the atmosphere.

Experiments showing that the germs of living organisms are present in the atmosphere.—With a view to actually demonstrating the presence of the germs of organisms in the atmosphere, Pasteur, after having inserted a plug of gun-cotton in a tube, drew a large quantity of air through it by means of an aspirator. He then dissolved the gun-cotton in a mixture of ether and alcohol, and examined the undissolved matter under the microscope. This was found to consist of minute particles partly of a mineral and partly of an organic nature; very frequently there were also present minute bodies which were undoubtedly the eggs of infusoria and the spores of fungi. The treatment with ether and alcohol unfortunately destroyed the vitality of

¹ The use of the cotton-wool for this purpose was suggested by some experiments of Löwel, who found that common air, after filtration through this material, was so far freed from solid particles as to be unable to cause the crystallisation of a super-saturated solution of sodium sulphate.

any of the germs present, hence it was impossible to study their further development

Experiments in which germs collected from the atmosphere were actually grown.—Sweetened yeast-water is a liquid particularly prone to undergo change when exposed to the atmosphere; if boiled for a few minutes and allowed to remain in an uncovered vessel for a few days, it invariably falls into a state of change, and is invaded with bacteria, torulæ, mould fungi, &c. Pasteur demonstrated that this liquid could be preserved indefinitely without undergoing change if calcined air only were permitted to come in contact with it. In order to demonstrate this, the necks of a series of flasks were each drawn out to a narrow tube, and a quantity of sugared yeast-water introduced into each flask. The neck of each of these was now connected in turn by means of india-rubber tubing, to a platinum tube maintained at a red heat. The contents of the flask were then boiled for two or three minutes, and, without being disconnected from the apparatus, its neck sealed up with the blowpipe. A series of flasks that had been treated in this way, and which were subsequently kept at a temperature of 30° C. (83° F.) for a considerable length of time,¹ did not show the slightest sign of change.

By an ingenious modification of the apparatus used in the foregoing experiments, a plug of ordinary cotton-wool on which atmospheric dust had been collected in a similar manner to that in which it had been collected on gun-cotton, was introduced into one of these flasks charged with the sweetened yeast-water which had been boiled for a few minutes, precautions being taken that nothing but calcined air obtained access to the plug or to the interior of the flask.

In a few days numbers of organisms made their appearance in the liquid similar to, but far greater in number than, those which would have appeared in the liquid had it been simply left exposed to the air. It is worthy of remark that the alcoholic fermentation was never met with in these experiments, though the liquid employed was one eminently adapted for undergoing this change. This series of experiments formed an effectual rejoinder to the objections which had been urged against the similar experiments of Schwann, viz., that the air could be so corrupted by incineration as to be incapable of starting life anew.

Liquids eminently liable to undergo change may be preserved for an indefinite period in free contact with atmospheric air, provided such air is freed from floating particles.—In order to demonstrate this, quantities of liquids liable to change, such as wort, grape-juice, &c., were introduced into flasks of convenient size (250 to 300 c.c.), the neck of each flask being afterwards drawn out to a fine tube and bent into the shape of a letter S. The contents of each flask were then boiled until steam freely issued from the orifice of the capillary tube, when the whole was allowed to cool. In this case, in

¹ This treatment is now technically known as "forcing," and small samples of finished beer are frequently tested in this manner to ascertain if they are contaminated with bacterial organisms.

addition to the liquid itself being sterilised during the boiling, the heated steam effectually sterilises the walls of the flask and the inside of the curved tube. As the temperature lowers during the subsequent cooling, air gradually enters to replace the steam, and any germs that it may happen to contain are caught and retained by the drop of condensation water which collects in the tube; they are in this way prevented from obtaining access to the contents of the flask. The tube eventually becomes dry, but the passage of air in and out of the flask is now so slow that its floating particles are all deposited on the side of the tube by gravitation. Organic liquids, such as beef-tea, grape-juice, &c., which are extremely liable under ordinary circumstances to enter into putrefaction or fermentation, and which are capable of being sterilised at a temperature of 100° C. (212° F.), may be preserved in such flasks for years. This form of flask was the precursor of the well-known Pasteur flask.

The Pasteur Flask.—In this, the form of the original flask is retained, but it has in addition a short straight tube inserted into its bulb, as shown in Fig. 56. This additional tube permits of additions to or withdrawals from the contents of the flask being made under suitable precautions, without running any risk of introducing foreign organisms. A suitable quantity of wort or other liquid having been introduced into the flask, a small piece of india-rubber tubing is attached to the side-tube, and the contents of the flask boiled. As the side-tube possesses the larger orifice, the steam rushes out of this first, effectually sterilising it and the india-rubber tube. When the boiling has proceeded for a few minutes the rubber tube is stopped by a piece of glass rod. The boiling is continued for a few minutes longer, and, as the steam is now compelled to pass through the fine curved tube, this is also sterilised. In order to prevent the chance of infection, which has occasionally been caused by insects crawling up the capillary tube and carrying germs with them, a small quantity of asbestos, previously ignited in the flame of a spirit-lamp or Bunsen burner, is inserted into the orifice of the fine tube just after the flask has been removed from the source of heat. When all these precautions have been fully carried out, the wort in such a flask will keep unaltered indefinitely. If there is any doubt that the sterilisation has not been perfect, the flasks so prepared may be easily tested by being “forced,” that is, kept for a few days at a temperature of 24° to 28° C. (75° to 83° F.). Such a temperature is exceedingly favourable for the growth and development of bacterial organisms, and in those extremely rare instances in which the sterilisation has not been perfectly attained the presence of organisms is readily detected by the alteration in the appearance of the liquid which ensues.

FIG. 56.—Pasteur Flask
(after Hansen).

Organic liquids, such as blood serum, urine, &c., when taken from a healthy

animal, may be preserved indefinitely without undergoing putrefaction if only allowed to come in contact with germ-free air.—The liquids contained in the various vessels and cavities of an animal when in a state of health are perfectly free from organisms, though, as is evinced by the extreme readiness with which they enter into a state of putrefaction under ordinary exposure to the air, they form excellent media for the growth and development of bacteria. It has also been conclusively shown that the blood of living animals does at times become invaded by organisms, and serious diseases are produced thereby. Pasteur demonstrated that liquids such as blood and various other secretions of the animal body could be preserved indefinitely in contact with air uncontaminated by germs in the following manner. A number of flasks, together with the air they contained, were thoroughly sterilised, they were then partially filled with blood taken directly from the blood-vessel of an animal—rigorous precautions being taken that the liquid suffered no contamination from the atmosphere during the operation—after which the flasks were hermetically sealed. Blood, which under ordinary circumstances exhibits the greatest proneness to putrefy, was kept in this way for several weeks at a temperature of 25° to 30° C. (77° to 80° F.) without showing the slightest tendency to putrefy. The same was shown to be true of many other organic secretions, and M. Gayon has since proved that it also holds good with reference to the contents of eggs.

These experiments form one of the greatest proofs of the fallacy of the theory of spontaneous generation, for they were made on substances which had been unaltered by heating; and they formed an effectual rejoinder to the arguments of some of the advocates of the theory of spontaneous generation, who had asserted, not without some show of reason, that materials which had been heated did not satisfy the conditions requisite for the spontaneous production of living from dead organic matter.

Tyndall's Experiment on the Floating Particles in the Air.—In 1881 Tyndall published the results of a remarkable series of experiments made at the Royal Institution, in which free exposure of highly putrescible liquids to the air was carried out in a still more extended manner.

Reasoning from the well-known fact that, when a powerful beam of light is passed through the air of a darkened room, its path is rendered distinctly visible because it illuminates the particles floating in that portion of the air which it traverses, he concluded that the path of such a ray would be invisible in an atmosphere which contained no floating particles. He argued that as all particles of matter, however small, must necessarily obey the law of gravitation and be precipitated in course of time, air, if confined in a close space and kept perfectly free from disturbance, should, after the lapse of a sufficient length of time, be entirely freed from all the floating matter which it originally contained. In order to put this to the test, Tyndall had a box constructed, the front and sides of which were glazed. After the apparatus had been left at rest for some time, his expectations were fulfilled, for on passing the powerful beam from an electric lamp through the box he

found its path invisible. He now reasoned that if the organisms which cause fermentation or putrefaction form a portion of the particles which float in the atmosphere, liquids liable to take on fermentative or putrefactive changes should, when placed in an atmosphere of this kind, remain unchanged for an indefinite time. In order to carry out this idea a very ingenious apparatus was constructed. It consisted of a four-sided box, in the front of which was fixed a plate of glass, the two sides of the box being similarly provided with smaller glass panes, whilst at the back of the box was a small door, so constructed that, when closed, the front, back, and sides of the apparatus were air-tight. The bottom of the box was perforated by several apertures into which fitted air-tight several large test-tubes. Passing through an aperture in the top of the box was a funnel with a long stem, fixed in such a manner that, whilst remaining completely air-tight, it could be moved about and its lower extremity placed in turn over the mouth of any individual test-tube. There were also inserted in the upper side of the box two tubes bent into the form of spirals. Before the apparatus was permanently closed the inside was smeared with a thin layer of glycerol, a liquid eminently adapted to retain any small particles coming in contact with it. The apparatus was now left at rest, until, on passing a beam of light from an electric lamp through it, no trace of floating matter could be observed; this was generally the case in about three days. The test-tubes were then partially filled by means of the funnel with various solutions, which under ordinary circumstances would have rapidly entered into a state of fermentative change; these were meat, game, and fish broths; decoctions of tea, vegetables, hay, &c. In order to destroy any germs which these liquids might contain, the lower portions of the test-tubes were immersed in a bath of heated brine and their contents boiled. The steam either condensed on the sides of the box or passed out of the bent tubes, and, after the heating, was replaced by atmospheric air, which entered by the spiral tubes. Treated in this way, liquids of the most putrefactive nature remained unchanged for months; when, however, the door at the back of the apparatus was opened for a few minutes and then closed, in a very short time some of the tubes began to exhibit signs of infection.

Here then was demonstrated the important fact that air, which resembled the outside atmosphere in every particular, with the single exception that it had been deprived of the floating particles which it originally contained, not by any violent means, such as passing it through a red-hot tube, or through sulphuric acid, or even filtering, but simply by natural gravitation, was unable to induce either fermentation or putrefaction in the most changeable liquids. Similar results were afterwards obtained by exposing solutions similar to the above to an atmosphere in which the organic particles had been burnt up by means of a spiral of platinum wire kept for some time in a state of incandescence by a current of electricity.

Organic Liquids of Varying Constitution require Different Temperatures to effect their Sterilisation.—It had been observed by investigators previous to Pasteur that, though most liquids were sterilised by exposure for a short

time to a temperature of 100° C. (212° F.), yet there were exceptions to this rule, and of these milk formed a notable example. Pasteur made numerous investigations as to the reason for this, and found that whilst the nature of the solution itself exercised some influence, the resistance to sterilisation chiefly depended upon the reaction of the liquid. Thus, acidity of the liquid favoured sterilisation, alkalinity retarded it. For example, the highly acid liquid vinegar was deprived of its liability to change by being subjected to a heat of not more than 50° C. (122° F.); grape-juice, which is but slightly acid, required a higher temperature, but not such a high one as unhopped wort, this, being still less acid, required a temperature of 90° C. (194° F.). Fresh milk, which is a neutral liquid, needed a temperature of 110° C. (230° F.); but sour milk needed only to be heated to 80° C. (176° F.) to prevent its undergoing further change. The real cause seems to lie in the degree of permeability of the exterior coats of the germs to moisture, acidity of the solution apparently permitting the ready penetration of moisture into the interior of the cells, whilst alkalinity has the reverse effect. The germs of the *Bacillus subtilis*, found by Tyndall in hay, were able to withstand the temperature of boiling water for several hours; this resistance he also considered to be due to some peculiarity in the external coating of the germs.¹

The state of dryness of an organism, or the reverse, is also of great influence in determining at what temperature it will succumb to heat; a mould fungus, which in a moist state cannot survive the temperature of boiling water, may, after thorough desiccation, be heated to a temperature of 120° C. (248° F.) without losing its fecundity. The same is true of yeast-cells, which, when perfectly dry, may be exposed to relatively high temperatures without losing their vitality.

Influence of the Acidity or Alkalinity of a Nutritive Liquid on the Nature of the Organisms which will develop in it.—Pasteur has shown that neutral liquids, or those having a slightly alkaline reaction, form media particularly favourable to the growth of bacteria and infusoria; a slightly acid medium, on the contrary, is unfavourable to the growth of most of them, but favours that of the mould-fungi and of the *Saccharomycetes*. A nutritive liquid when exposed to the air becomes contaminated with organisms of all kinds; much, therefore, depends upon its reaction as to what shall be the class of organisms that will flourish in it or be suppressed. When a liquid, such as grape-juice, which has a strongly acid reaction, is exposed to the air, the germs of the bacteria which fall into it are suppressed; whilst those of the moulds and *Saccharomycetes* flourish. If, however, the grape-juice is neutralised before being exposed to the air, then bacteria will flourish to the exclusion of the other organisms, for when once one particular set of these has taken

¹ Instances of the same nature are occasionally met with in the seeds of the more highly organised plants; for instance, Pouchet (*Bot. Zeit.*, 1878, p. 153) found that the seeds of a certain kind of lucerne, which adhere to the wool of sheep imported into France, were able to germinate after being boiled in water along with the wool for four hours.

possession of the field it is very difficult for others to obtain a footing. Under such circumstances, neutralised grape-juice, instead of undergoing alcoholic fermentation, putrefies. The slight acidity of ordinary beer-wort has considerable influence not only in assisting its sterilisation in the copper, but also, to some extent, in guarding it against the invasion of foreign organisms during the processes subsequent to the boiling.

The difference which the nature of the organic liquid exercises in favouring the development of particular organisms lent considerable support to the theory of spontaneous generation. When an organic liquid, such as grape-juice, is exposed for a time to the atmosphere, it is invaded by the germs of all kinds of organisms, but owing to its slightly acid nature the growth and development of bacterial organisms are suppressed; *Saccharomycetes*, which thrive in a slightly acid medium, on the other hand, flourish. Consequently, yeast may be grown over and over again in a slightly acid medium, such as grape-juice, its reproduction not being interfered with by the acidity of the medium; but if the yeast was originally impure, for instance, contaminated with any of those organisms which turn wine sour, the growth of these will be so checked in proportion to that of the yeast, that their number becomes relatively so small in comparison with that of the yeast-cells that they may easily escape detection by a microscopical examination. Under such circumstances, by assuming that such a yeast is pure, the mistake may readily be made that yeast can be transformed into the organisms which turn wine sour, if it is not tested by direct experiment for impurity.

This is the error into which M. Duval evidently fell when he imagined that he had demonstrated that yeast could be transformed into lactic ferment. By adding yeast (which he considered to be pure, since he had cultivated it many times in grape-juice) to a mixture of sour milk, glucose, chalk, and ammonium phosphate, he readily obtained the lactic ferment, and the product of its action, lactic acid, in the form of calcium lactate. As Pasteur points out, though Duval supposed that his yeast had been freed from foreign organisms by growing it repeatedly in grape-juice, yet he had not put it to the test of direct experiment, in order to ascertain with absolute certainty that it was pure.

The varieties of the different organisms which are found to develop in liquids which have been sterilised by heat are comparatively few in number, since they can only be those which have obtained access to the liquid from the atmosphere after its sterilisation; obviously all the germs with which the liquid was previously contaminated, whether derived from the atmosphere, from the dust deposited on the containing vessels, or from the materials employed, are destroyed in the process of heating. The organisms which develop in such a liquid are not nearly so numerous as has been imagined, since, unless in a state of violent agitation, it can only hold in suspension the finest particles. All observations which have been made on spontaneous generation have been made in rooms or laboratories where the air is comparatively quiescent; consequently, the variety of organisms which would

make their appearance in sterilised liquids after exposure to the atmosphere of such localities is limited in number. This is still further lessened because some of the germs which do find access to the liquid are suppressed by competition. That such is the case is shown by the fact that a much larger variety of organisms is found when a sterilised organic liquid is exposed for a short time to aërial contamination, and then immediately divided into several portions, each of which is closed up in a separate vessel. There is then the chance that only one or two species are enclosed in one vessel; and consequently there is not the same chance of suppression by competition.

The variety of the organisms which make their appearance in raw infusions of different organic substances, such as leaves, fruits, grass, &c., is much greater in number, since the substances carry with them the germs of larger organisms deposited from the air when it has been in a state of violent movement, and, in addition to these, plants are always more or less infested with minute parasitic organisms.

Competition amongst the Organisms introduced into a Fermenting Liquid.—The fact that when a large number of organisms of a certain species are added to a fermenting liquid—such, for instance, as the addition of yeast, which consists principally of *Saccharomyces cerevisiæ*, in fairly large quantity to beer-wort—other organisms, such as the acetic and lactic bacteria, together with the germs of other *Saccharomycetes* which may have accidentally obtained access to the wort, and which are comparatively few in number, are kept in subjection by the preponderance of the *Saccharomyces cerevisiæ*; they are, as it were, crowded out, or, in other words, lose in the struggle for existence, when the yeast is present in large quantity. The brewer seeks in this way, by adding sufficient vigorous yeast, to rapidly induce alcoholic fermentation in his wort, and in this way keep in subjection those other organisms which would exert a prejudicial action on it. It must, however, be borne in mind that the deleterious organisms are not destroyed, they are merely suppressed, and when the alcoholic ferment has exhausted its course, then these inimical organisms are able to commence their functions. Thus, when the germs of the acetic ferment have obtained access to a wort, and though during the progress of the alcoholic ferment they have been held in check, yet, when that process is completed, they will then begin to manifest their presence by the souring of the beer. This peculiar property that one ferment possesses, of when in abundance suppressing by competition other ferments which occur in less numbers, has led to erroneous views being held, by some observers, who, finding that as one ferment became exhausted and died off another of a different nature took its place, argued that one ferment had become transformed into the other.

Transformation of One Set of Organisms into Another.—Numerous observers, commencing with Turpin, and including Bail, Hoffmann, Hallier, Trecul, Robin, and Fremy, believed they had actually observed the transformation of one set of ferment organisms into another. One of the latter of these, M. Trecul, stated that he had seen this transformation take

place in unhopped wort prepared at a temperature of from 60° to 70° C. (140° to 160° F.). First, a number of fine granules appeared in the wort, these developed into active bacteria, which afterwards became quiescent and were then transformed into the lactic ferment. After a time larger granules made their appearance, which gradually increased in size, and were eventually transformed into globular and elliptical cells almost as large as yeast-cells; these, after a time, commenced to bud. He also asserts that he has sown the spores of the *Penicillium glaucum* in boiled wort and obtained from them a *saccharomyces*.

Pasteur pointed out that when wort is boiled in one of his flasks and allowed to stand for some time, though an abundance of granular deposit invariably forms, in no case is it transformed into a living organism. He then demonstrated that M. Trecul must, unintentionally, have introduced other germs along with those he thought he had sown in a pure state, for when a sterilised liquid is impregnated with a pure culture of any individual organism, nothing but that organism is ever reproduced.

J. Juhler in 1895 stated that he had observed that the conidia of *Aspergillus oryzae* (see p. 288), when this mould develops the power of saccharifying starch, are transformed into yeast cells which are capable of producing alcoholic fermentation and exhibit all the properties of the typical *Saccharomyces*. Jörgensen following up this observation came to the conclusion from his own experiments that certain mould fungi which occur on grapes, after passing through various transition forms, finally evolve the wine yeast *Saccharomyces ellipsoideus*. Doubt was thrown from the first on these alleged transformations by Hansen and finally the experiments were repeated with negative results by Klöcker and Schöning.

Methods of Obtaining a Pure Culture.—For the purpose of securing a pure culture of an organism Pasteur employed the following two methods. In the first of these, a convenient-sized flask was partially filled with wort, its neck drawn out to a fine tube, and its contents boiled. While steam was issuing from its orifice, the tube was hermetically sealed up with the blow-pipe. On cooling the flask the steam gradually condensed, and a partial vacuum was formed. When the point of the tube is broken off air rushes in to fill the vacuum, carrying in with it any germs it may happen to contain. Sometimes the air admitted into a flask in this way contains the germs of several different organisms, as is shown by their subsequent development; at other times no germ is introduced, and the contents of the flask remain sterile. Occasionally only a single germ obtains entrance, and in this last case a pure culture is obtained, which may be used for impregnating the contents of an ordinary Pasteur flask containing sterilised liquid. In the second method, which Pasteur says is nearly as reliable as the first, the growth of a mould fungus is allowed to proceed on any object on which it may have adventitiously formed until it has produced a crop of spores. A piece of recently ignited platinum wire is then passed lightly over the fructifying heads of the fungus, when a few spores adhere to the wire. This with its adherent spores

is then introduced into a Pasteur flask containing a sterilised solution. The spores germinate and are allowed to grow on the surface of the liquid until they have produced plants, from which some of the spores may be removed by means of a platinum wire and used in turn in a similar manner.

The Origin and Distribution of the Germs present in the Atmosphere.—Now that it had been conclusively proved that fermentation and putrefaction were invariably caused by organisms present in the floating matter of the atmosphere, the origin and distribution of these in the air of different localities became a matter of considerable interest and importance. This subject has been ably investigated by Pasteur and Hansen, who, however, chiefly directed their attention to the organisms of fermentation found in the atmosphere; whilst other observers, such as Miquel, Petri, Frankland, and many others, interested themselves principally with the bacterial organisms that it contains.

Pasteur's Examinations of the Air of Different Localities for Organisms.

—For this purpose Pasteur employed flasks, prepared in exactly the same manner as those described at p. 321 for obtaining a pure culture of a mould fungus. A number of these were taken to the locality in which the nature of the organisms in the air was to be investigated, the points of the flasks were broken off in turn, and each immediately sealed up again. They were then brought back to the laboratory and kept at a forcing temperature for some time. The general appearance of the organisms which developed in them was noted, and a rough estimate of their number made.

In an experiment of this character performed at the École Normal, ten flasks containing sterilised grape-juice were opened at the bottom of the garden; another ten on the landing of the second floor of the building; and a third ten in the laboratory itself, where experiments on fermentation were being constantly carried on. Of the first ten flasks only one was invaded by organisms; of the second ten, four were similarly affected; and organisms developed in all those belonging to the third set. The difference in the number of germs contained by the same amount of air in these three different localities was therefore considerable. The nature of the germs also differed; the first two series of flasks contained mould fungi only, whilst those taken in the laboratory contained *Torulæ* (p. 265) in addition to mould fungi.

Experiments of a similar nature were made upon the air of the most diverse localities, and it was found that germs were very abundant in the air of towns, but that they gradually decreased in number as the locality became more and more remote from human habitation. Of twenty-four flasks opened on Mount Poupet, at an elevation of 2800 feet above the level of the sea, only five were infected. Of another twenty, opened at Montanvert, near the Mer de Glace, 6560 feet above sea-level, when a strong wind was blowing from the glacier, only one was infected. In experiments made in the higher regions of the atmosphere by means of a balloon, the air was found to be absolutely free from germs.

Ferment Organisms or their Germs are seldom present in the **Atmosphere**.—But, said Pasteur's opponents, if it were true that fermentation was invariably caused by the germs present in the atmosphere, then they would be present in the air in such countless numbers that they would form a thick black fog as dense as iron; for it is a well-known fact that when grapes are crushed in any part of the world, even on the top of a high mountain, their juice invariably enters into fermentation. In reply to this, Pasteur demonstrated by a series of experiments that the germs of the fermentation organisms do not occur in extraordinarily great numbers in the air of the atmosphere.

It was a very rare occurrence to meet with fermentation organisms in the experiments just mentioned, or in those performed with plugs of cotton-wool on which the dust of the atmosphere had been collected. They were only found in appreciable quantities in the air of rooms where fermentations were being constantly carried on. For instance, out of seven flasks opened in Pasteur's principal laboratory, where this condition obtained, only one exhibited signs of alcoholic fermentation. This fact was further demonstrated by the following experiments: A number of bottles of wort that had been preserved by Appert's process were uncorked, all necessary precautions being taken to prevent their contents being contaminated by the dust deposited on the cork, neck of bottle, &c., and allowed to remain undisturbed in various places. Two of these were exposed in an underground room of the École Normal, where experiments on fermentation were continually being carried on. The contents of the first were found to have fallen into a state of active fermentation in six days, and the second, which up to the sixteenth day had shown no sign of fermentation, was in active fermentation on the twenty-third. Of four bottles exposed for three months in a room only occasionally used for experiments in fermentation, the contents of none entered into fermentation. The contents of four bottles placed in an incubating chamber where fermentations were constantly in progress, and allowed to remain there five months, showed no sign of fermentation. Of ten bottles that were exposed in another room where fermentation experiments were continuously going on, the contents of one of them commenced to ferment in four days; the day after, those of another started; and in fourteen days two more. All the remainder had up to this time shown no signs of fermentation, and these after each had been covered with a cap of sterilised paper, were kept under observation for three months. The contents of all these last failed to enter into fermentation.

Fermentation Organisms or their Germs are extremely abundant on the **Skins of Ripe Fruits**.—Pasteur showed that ferment organisms were always present in great abundance on the exterior surfaces of ripe fruits. He picked a bunch of grapes from the vine, cut off each grape with a pair of sterilised scissors, washed it and the little stalk attached to it with a small quantity of water by the aid of a perfectly clean badger-brush. A dozen grapes successively washed in this way with 3 c.c. of water yielded a turbid mixture, which,

when examined under the microscope, was found to abound with minute organisms. The development of these was studied, and it was also proved that among them were some that were capable of inducing alcoholic fermentation. This was also found to be the case with ripe gooseberries, plums and pears. The woody portion of a bunch of grapes, from which the grapes had been detached, was similarly treated, and the number of organisms was found to be even greater on this than on the skins of the grapes themselves. In another series of experiments he proved that the organisms of fermentation were only present on the surface of the grapes and their stalks in the autumn, when the grapes were fully ripe; that they were not to be found on the immature fruit, and that grapes kept through the winter gradually lost their power of causing fermentation.

Pasteur also made a series of experiments in order to show that the organisms of fermentation were situated on the outside of the grape alone, and did not exist in the juice in its interior, and also to explode the hypothesis of semi-organisation propounded by M. Fremy in 1864, and subsequently adopted and extended by M. Trecul in an attempt to explain the formation of yeast from the albuminous portions of grape-juice. By this hypothesis it was assumed that organic bodies, such as albumin, casein, fibrin, &c., which have an extremely complex chemical constitution, were in reality semi-organised substances; that is to say, they held a position intermediate between dead and living matter. These semi-organised bodies," says M. Fremy, "which contain all the elements of organs, have the power, like a dry seed-grain, of existing in a state of organic immobility, and of becoming active under circumstances which favour organic development. By reason of the *vital energy*¹ which they possess, they undergo a succession of decompositions, giving rise to new derivatives and to the advent of ferments, not by any process of spontaneous generation, but by a process of vital energy which pre-exists in the semi-organised bodies, and is simply carried on, when this energy manifests itself in these most varied organic changes."

Forty Pasteur flasks were taken, and a portion of filtered grape-juice introduced into each of them. The contents of each flask were then boiled for a few minutes to effect sterilisation, after which the flasks were divided into four groups of ten each. The first group was kept without further treatment as a check on the experiments. Part of a bunch of grapes was washed with a few c.c. of water, and to each flask of the second group a few drops of this were added. To each flask of the third group a similar quantity of the water in which the grapes had been washed was added after it had been boiled. To each flask of the fourth group a single drop of grape-juice obtained from the interior of a grape was added, the experiment being so arranged that none of the organisms present on the exterior of the grape could obtain access to the drop. The whole series of flasks was then kept at the ordinary summer temperature. For some time the contents of the flasks belonging to the first, third, and fourth groups showed no change. All those

¹ A purely gratuitous assumption, Pasteur remarked.

of the second were found, at the end of forty-eight hours, to be in a state of fermentation, and in some of these the mycelia of mould fungi afterwards appeared; in others *Mycoderma vini*, but in no case bacteria, since these last would be suppressed by the liquid as it had an acid reaction. Obviously, if ferments could be formed from the albuminous substances present in grape-juice, as affirmed by Mons. Fremy and Trecul, the contents of every one of the flasks ought to have fermented. It was also shown that occasionally fermentation organisms were entirely absent on the surface of a grape; consequently, such a grape might be crushed without its contents fermenting—a fact that also militated against M. Fremy's theory.

The organisms which spontaneously cause changes in organic liquids are not, as a rule, derived directly from the atmosphere, but indirectly from it in the shape of dust which has been deposited on vessels and other objects with which the liquid comes in contact.

That this is so is well exemplified in Pasteur's modification of the experiment of Gay-Lussac, mentioned on p. 312, which consists of uncorking a bottle of grape-juice, preserved by Appert's method, and transferring its contents to another bottle, when the liquid so transferred invariably falls into a state of fermentation. This fact was adduced by Gay-Lussac as a proof of his theory that it was the action of the oxygen of the atmosphere on the albuminous constituents of the liquid which caused its fermentation. Pasteur repeated the experiment, and obtained the same result when no precautions were taken to ensure that the liquid was not contaminated by the dust on the cork and on the outside of the bottle; but when scrupulous precautions were taken, such as singeing¹ the cork and neck of the bottle, the corkscrew used, and taking care that the bottle into which the liquid was introduced was perfectly free from germs, no sign of fermentation made its appearance.

A Fermentable Liquid may be preserved for Years in an ordinary Pasteur Flask, but if a little of the dust which has become deposited on its exterior be introduced into the liquid, change, indicated by turbidity, will manifest itself on the following day. The same object may be effected by tilting the flask so that its liquid contents come in contact with the lower portion of the capillary tube, where germs are invariably deposited; or even violently shaking the flask so as to disturb the deposited germs by a violent rush of air into the capillary tube is sufficient to infect the liquid.

Even the mercury sometimes employed in experiments of this nature may contain the sources of infection. This was exemplified in Pasteur's explanation of the fallacy involved in an ingenious experiment performed by M. Pouchet. In order to show that organisms could develop in a liquid which had never come into contact with ordinary air, M. Pouchet filled a bottle with

¹ This ingeniously simple method of sterilising small articles, such as forceps, scissors, &c., just before being used, is due to Pasteur. It is effected by passing the articles through the flame of a spirit-lamp or Bunsen burner; owing to the extreme readiness with which minute organic particles ignite, any germs deposited on such articles are immediately consumed.

boiling water, then plunged it into, and uncorked it, under mercury. When the water was cold, half a litre of oxygen gas was introduced, and a portion of hay which had been exposed for several hours to a temperature of 300° C. (572° F.) was passed under the mercury into the neck of the bottle. At the end of a week's time organisms appeared in the liquid. Pasteur showed that here the source of error lay in the particles of atmospheric dust with which the mercury was contaminated. By passing a beam of light through the room after being darkened, he was able to demonstrate ocularly to his audience the particles of dust which were continually floating about in the atmosphere, and although these were apparently moving about in all directions yet each one had the general tendency finally to settle down by gravitation. The surface of mercury which is frequently exposed to the atmosphere would undoubtedly receive its share of these particles; and it was clearly shown, by throwing a strong light on to the surface of perfectly clean mercury, spreading some dust on this, and plunging a glass rod into the metal, that the dust travelled to the spot where the rod was inserted, and was actually carried into the interior of the quicksilver.

Miquel's Experiments on the Floating Matter of the Air.—In these investigations, which were carried out at the observatory of Montsouris near Paris, Miquel employed, at first, an apparatus which he called an "aëroscope." In this the air to be examined was caused to impinge in a fine jet on a glass plate smeared with a mixture of gelatin and glucose, which, from its sticky nature, was eminently adapted to catch and retain the floating particles which the air contained. These particles were then evenly distributed over the glass plate, placed under the microscope, and counted. Since this method gave no information as to the number of living organisms contained in a measured quantity of air, he devised a peculiarly constructed flask in which a measured quantity of air was caused to bubble through a known quantity of water, certain precautionary measures being taken to avoid loss of organisms. In this way he obtained the dust of the atmosphere suspended in water, which, after being diluted with a further quantity of sterilised water, was added in small and equally measured portions to small quantities of sterilised broth contained in a number of flasks. When a large proportion of the contents of the flasks treated in this way exhibited no signs of infection, there was a considerable probability that each of the infected flasks contained only a single germ; consequently, by taking into account the quantity of air passed through the apparatus, and the number of flasks which became infected, it was possible to form an approximate estimate of the number of organisms that were present in a sample of air capable of development.

Koch's Method.—An entirely different form of apparatus was employed by this investigator. It consisted of a glass tube about a yard long and two inches wide. One end of the tube was closed with a perforated india-rubber cork, a glass tube plugged with sterilised cotton-wool being inserted in the aperture in the cork. A quantity of sterilised gelatin meat-broth was then introduced into the tube and its other end closed with an india-rubber cap.

The whole was then sterilised, laid on its side, and allowed to cool. When about to be used, the glass tube was connected with an aspirator, the india-rubber cap removed, and air drawn slowly through the apparatus. In its slow passage through the tube any particles which the air might happen to contain would be gradually deposited on the gelatin. The india-rubber cap was then replaced on the end of the tube, and the whole transferred to an incubator, where it was allowed to remain until the organisms had developed and formed colonies. These were then counted and their general appearance noted. A curious fact was noticed in experiments conducted in this way; the bacteria which had developed were found in that portion of the tube nearest the end where the air entered, whilst the mould fungi were found much farther down the tube; this seems to show that the specific gravity of the germs of the former organisms is higher than that of the germs of the latter.

Hueppe's Method.—In this, the air under examination was caused to bubble slowly through warm sterilised gelatin meat-broth, which was afterwards spread upon glass plates. These were placed in an incubator, and the colonies that formed on them studied.

Method of Frankland, Miquel, and Petri.—In this, the floating particles of the air were collected by passing it through filters containing such substances as sterilised sand, powdered glass, glass wool, &c. The material of the filter was afterwards mixed with sterilised gelatin meat-broth and spread on glass plates; or the sand, &c., with the germs it contained, were placed in a Petri dish, intimately mixed with gelatin meat-broth, covered over, and placed in the incubator.

Objections to the use of Gelatin Meat-broth in Experiments of this Nature.—As was shown by Miquel, there are several objections to the use of gelatin plates for this purpose. They cannot be used at a temperature higher than 24° C. (75° F.), since above this the gelatin medium becomes fluid, and some bacteria require a higher temperature than this for their development. Many bacteria take a fortnight to form colonies at the usual temperature of the incubator, and by this time so much of the plate is liquefied by the action of certain bacteria, or the plate may be so covered over with the rapidly growing mould fungi, as to be unfitted for further observation; consequently, those organisms which have not had time to develop entirely escape observation. Some species of organisms which readily develop in liquids will not do so on a gelatin plate. Finally, there is no absolute certainty that one colony only represents one species of organism. This was demonstrated by growing colonies on a plate in the ordinary way, then sowing each of them in a separate batch of meat decoction. A second plate culture was afterwards made with each batch of the infected broth; and, as in these latter colonies species were found which differed from those of the original colony, distinct evidence was afforded that the original colony by no means consisted of one species.

Hansen's Investigations of the Air.—These experiments made between the years 1877 and 1882, were carried out with the object of ascertaining the

nature and distribution of those organisms in the atmosphere which might exert a prejudicial effect on the operations of the brewer. For this reason, as in his method of examining water used for brewing purposes, he entirely rejected the use of gelatin, and used the two liquids with which the brewer is mostly concerned, *i.e.*, wort and beer. Two forms of apparatus were employed in the course of these experiments: first, the vacuum flasks (p. 327) introduced by Pasteur, only, instead of sealing up the ends of the tubes by fusing the glass, Hansen simply closed them with sealing-wax; the second consisted of ordinary boiling flasks partially filled with wort, which were sterilised after their necks had been covered with filter-paper. The flasks were taken to the place the air of which was to be examined, their caps removed, and allowed to stand open for various periods of time, in some cases as long as forty-eight hours. When using the vacuum flasks, it was found that the criterion for infection used by Pasteur, namely, a visible alteration in the contents recognisable by the naked eye, was not to be implicitly relied upon in all cases, for small quantities of various growths, such as those of *Aspergillus*, *Penicillium*, *Mucor*, *Cladosporium*, *Bacterium aceti*, and *Mycoderma cerevisia*, could only be detected by microscopical examination. He confirmed Pasteur's observation that it was often possible in this way to obtain a pure growth of one particular organism, a single germ only having obtained access to the flask; in fact, it was only in rare instances that several growths were obtained in the same flask. An observation made in this way necessitates the employment of nearly one flask for each germ present in the air examined, and although it gives the most certain information, it is exceedingly tedious and cumbrous in its application; it has the further disadvantage of only giving information as to the germs present in the air at the particular moment of opening each flask. Consequently, it was employed in conjunction with the second method, since this gave information as to the characters of the germs which appeared in the air during a longer or shorter period.

General Conclusions obtained from these Observations.—All these experiments tended to confirm the previous conclusions of Pasteur, that the air of places in immediate proximity to one another may contain at the same time not only the most diverse organisms, but also the most variable quantities of them. The germs of organisms seem to exist in the atmosphere in the form of clouds, the intervening spaces between which are comparatively germ-free. The number of bacterial organisms present in the air is much modified by the weather; they are most abundant in dry seasons, whilst after a spell of wet weather their number considerably diminishes. The reverse seems to be the case with the mould fungi; these occur in the greatest number in the atmosphere in damp weather, and in much smaller quantity in dry weather. *Saccharomycetes* are seldom met with; Hansen found that the yeasts increased in number from June to August, and are most abundant at the end of August and the beginning of September (the period of the ripening of fruit), after which their numbers again decrease. As there is strong evidence that the *Saccharomycetes* have their place of abode in the earth.

during the periods when they are not living on the surface of fruits, they are probably met with most frequently in the air when passing through it to change their respective resorts, viz., the ground and the fruit-trees. During this same period bacterial and mould fungi occur in the atmosphere in the greatest numbers, and hence it is at this season that the wort, when on the coolers and refrigerators, is likely to receive its greatest contamination from these organisms. This time of the year is proverbially known to be the worst for brewing operations.

Hansen's Investigations on the Air of Breweries.—Hansen now turned his attention to an examination of the air of the various rooms where the actual brewing, or the operations connected therewith, were carried on. The air of a passage leading to a room where barley was turned, as might readily be expected when the large number of organisms always present on its surface is taken into consideration, was found to be highly contaminated with organisms, especially those of a bacterial nature. The air in the neighbourhood of malting-floors was found to be extremely rich in mould fungi. The air of the fermenting-rooms of the old Carlsberg brewery was found to contain fewer organisms than that of any of the other localities hitherto examined; in that of the new Carlsberg fermenting-rooms organisms were comparatively abundant, from 55 to 100 per cent. of the flasks employed becoming infected.

In the case of the old Carlsberg brewery the air is freed from germs by passing it through a bath of salt water, and by rigorous attention to the cleanliness of the room and all the utensils contained therein.

Morris ¹ has recorded the results of a series of experiments made to ascertain the number of organisms that fell from the atmosphere into the wort during the time it was standing on the coolers. The season of the year is not given. These experiments were made at two different breweries; the cooler of the first had a superficial area of 70 square yards, that of the second an area of 426 square yards. It was found that rather more than 140,000 organisms fell on each square yard of cooler surface per hour, the numbers being pretty nearly equal in both breweries. He also determined the number of organisms with which the wort had been infected during its stay on the coolers in the following manner: wort was taken from the coolers when nearly the whole had run off and only a few barrels remained; this was added in measured portions, firstly, to 5 per cent. sterilised gelatin; secondly, to 5 per cent. sterilised meat-extract-gelatin. The number of colonies which had developed in three days were counted, and the quantity thus found calculated into wort per barrel. In the first brewery 163,548,000 colonies per barrel developed in gelatin, and 16,354,800 in meat-extract-gelatin. In the second brewery 1,406,512,800 developed in gelatin, and from 14,065,128,000 to 21,097,692,000 in meat-extract-gelatin. This great difference in the respective numbers was attributed to the difference of the superficial area in the two coolers. Of the organisms which developed in gelatin alone, the majority consisted of *Saccharomycetes* and *Torulæ*, though bacteria and mould fungi

¹ *Trans. Lab. Club*, vol. 3, p. 36.

were present in considerable numbers as well. The utmost cleanliness was observed in both breweries, and there can be no doubt that all the organisms found were derived solely from the air.

Yeast may be dried and reduced to a fine Powder without losing its Vitality.—Pasteur made the following experiments,¹ to show that yeast may exist in a state of vitality in the form of dust floating about in the atmosphere. A few grammes of pressed yeast were rubbed up with five times their weight of sterilised plaster of Paris in a sterilised mortar. The mixture was then wrapped in a piece of sterilised paper and dried at a temperature of 20° to 25° C. (68° to 77° F.). Two days afterwards a pinch of this powder was sown in sterilised wort, contained in a Pasteur flask, and the whole kept at a temperature of 20° C. (68° F.), when, in three days, signs of fermentation made their appearance in the wort. When the yeast was two and a half months old the experiment was repeated; this time fermentation commenced on the fourth day, hence the vitality of the yeast was not destroyed, but merely somewhat lowered. In a similar experiment made with the same yeast when seven months old, the vitality was not yet altogether lost, but was still more depressed, for it took eight days for signs of fermentation to appear.

At the end of ten months the yeast, when again tested in this manner, was found to be completely dead; the wort impregnated with it, though observed for several months afterwards, gave no sign of fermentation.

Pasteur showed that it was quite possible artificially to impregnate the air of a room with yeast organisms. In order to effect this it was only necessary to drop a small quantity of the dried yeast powder from a height, when the presence of living yeast in the air of the room could be demonstrated by opening a series of vacuum flasks in it. It often happened in experimenting in this manner, that one flask received but one organism; and in this way a pure culture of a single species of yeast was obtained, since the whole of it must have sprung from one mother-cell. At the conclusion of the description of these experiments, Pasteur adds the following pregnant sentence: "Our preliminary observations, although incomplete, seem to favour the idea that numerous varieties of ferment are to be obtained by these means." Here is distinctly foreshadowed the important field of investigation which has been, in later years, so ably worked out by Hansen.

Pasteur's Experiments on Fermentation.—From what has gone before, it will be remembered that ever since the discovery had been made that yeast was a living organism, the opinion had been held by various observers that fermentation was intimately connected with the vital functions of the yeast. Pasteur was, however, the first to put this into a definite shape, and to attempt to give a really scientific explanation of the processes of fermentation. In studying the peculiar fermentation which impure calcium tartrate entered into in hot weather when allowed to remain under water, he was led to conclude that the fermentation was caused by a small living organism.

¹ "Studies in Fermentation," p. 81.

Struck by the part played by this organism in the fermentation of tartaric acid, he thought that it could not be an isolated fact, but that it must form a portion of some great law, the elucidation of which could not fail to throw considerable light on the hitherto obscure problem of fermentation. He regarded fermentation and putrefaction as analogous processes, and recognised in them Nature's method of converting the dead refuse material of the animal and vegetable world into forms again available for the nutrition of plants.

Investigations on Lactic Fermentation.—When a mixture of milk, sugar, putrid cheese, and chalk¹ is kept in a warm place, it speedily falls into fermentation, and in course of time lactic acid is produced in abundance, according to the formula :

The acid unites with the chalk to form calcium lactate. Hitherto no organisms had been detected in such a fermenting mass ; Pasteur, however, showed that they were there, and that they were merely hidden by the innumerable host of other particles present in such a mixture, since they could occasionally be detected in the grey scum which formed a zone on the surface of the mass. In order to obtain the ferment in a state of purity, a decoction of yeast, to which sugar and calcium carbonate had been added, was infected with a minute trace of this grey scum. Fermentation ensued, the calcium carbonate was gradually transformed into calcium lactate, and the solution gradually grew turbid from the formation of the lactic ferment. This, when examined under the microscope, was found to consist of minute bacteria.

Experiments where the Albuminous Material was replaced by an Inorganic Nitrogenous Body.—In order to demonstrate the complete fallacy of the views held by Liebig and his followers, Pasteur repeated the foregoing experiment, employing, instead of the albuminous material derived from the yeast, an organic salt of the inorganic nitrogenous substance ammonia together with a small quantity of the phosphates of potassium and magnesium as mineral food. The lactic ferment multiplied and caused fermentation just as in the previous case. An experiment was also conducted with a liquid of similar composition, but to which, instead of lactic ferment, a minute trace of beer-yeast was added. A similar growth and multiplication of the yeast-cells took place, accompanied with alcoholic fermentation. Evidently, then, this was a plain proof that yeast could assimilate the inorganic substance ammonia, and derive from it the nitrogen necessary for forming its albuminous constituents ; the phosphates supplied the mineral food, and the carbonaceous

¹ A good mixture for preparing lactic acid is made up as follows : Skimmed milk, 2 gallons ; raw sugar, 6 lb. ; water, 12 pints ; putrid cheese, 8 ounces ; chalk, 4 lb. The mixture is kept at a temperature of 30° C. (86° F.) in an open vessel, and occasionally stirred. The lactic fermentation will be finished in about three weeks. The mass thus obtained is drained, pressed, and purified by recrystallisation in water, and oxalic acid added to remove the calcium from the calcium lactate. This leaves lactic acid in solution which may be further purified by conversion into the zinc salt,

portion was derived from the sugar. In these cases there was not the slightest possibility of fermentation being produced according to the theory of Liebig, for there was no albuminous matter present except that actually contained in the yeast itself; and to be certain that the albuminous material of the new yeast-cells could not be obtained from old and dying yeast-cells only a minute trace of yeast was added at the outset. Here, too, it was demonstrated that fermentation is a phenomenon of nutrition, during which the yeast is able to build up the complex bodies which enter into its composition from sugar and purely mineral substances.

The Ferment of Butyric Acid.—If, in the process mentioned above for the production of lactic acid, the action is allowed to proceed for a considerable length of time, the calcium lactate is gradually transformed into calcium butyrate, carbon dioxide and hydrogen being evolved, according to the formula :

This has led to the idea that the lactic acid ferment was gradually transformed into the butyric acid ferment. Pasteur demonstrated that the two ferments exhibited important distinctions; the lactic ferment is motionless, the butyric is in a constant state of lively motion. The latter ferment is also distinguished by another extraordinary property; it is able to live and perform its functions in the entire absence of free oxygen; in fact, its vitality is destroyed by that gas. If a current of air be passed for a few hours through a liquid undergoing the butyric fermentation, the process is completely arrested, the bacteria cease all active movement and fall to the bottom of the vessel. When sown in a liquid capable of undergoing the butyric acid fermentation, the ferment increases sensibly in weight, though, as with the rest of the fermentation organisms, this is exceedingly small when compared with the amount of substance decomposed.

This important discovery led Pasteur to divide organisms into two classes, the first, which are only able to live when supplied with free oxygen, he designated "aërobic"; the second, which are only able to exist in its entire absence, he styled "anaërobic."

Fermentation a Phenomenon of Nutrition.—Pasteur having decided in his own mind that fermentation was a phenomenon of nutrition, the next question for elucidation was wherein it differed from the ordinary process of nutrition. As a rule, an organism assimilates nearly the whole of the food which it absorbs, but in the case of a fermentation organism the amount of substance absorbed and decomposed is enormously greater than the small portion which it appropriates for its own nutrition. The fact that the butyric acid bacteria lived and could perform their functions in the absence of free oxygen struck him as probably furnishing the key to the mysterious problem of fermentation. Perhaps it was only the anaërobic organisms which were able to functionate as ferments, the aërobic ones being incapable of doing so. He conceived that there might yet be a third class of organisms represented

by the yeast fungi, which had two phases of existence, the one aërobic, the other anaërobic. In their aërobic state of existence they would live and carry on their nutritive functions after the manner of ordinary fungi; in the anaërobic they would act as ferments. The following observations and experiments seemed to confirm this view.

Several of the Mould Fungi are able to act as true Ferments.—In 1857 M. Bail had discovered that the mould fungus, *Mucor mucedo*, was able to excite alcoholic fermentation; this he wrongly attributed to the transformation of the fungus into yeast. Pasteur afterwards found that several of the mould fungi, when entirely submerged in a saccharine liquid, that is, when they were deprived of free oxygen, were able to produce alcohol and evolve carbon dioxide; in other words, to act as true ferments. His first experiments were made with *Aspergillus glaucus*.¹ A pure culture of this was sown on the surface of wort contained in a Pasteur flask, the experiment being so arranged that the little plant was supplied with an abundance of air. It then grew after the ordinary manner of a mould fungus, that is, absorbed oxygen and exhaled carbon dioxide, and decomposed no more sugar than was necessary to supply the requirements of its own nutrition. When the solution on which it had been growing for some time was distilled, it was found not to contain the slightest trace of alcohol.² When, however, in a similar experiment the plant was deprived of air by being submerged in the liquid, its general appearance and method of existence suffered great alteration, the hyphæ became swollen to some six times their natural thickness, and assumed a singular contorted appearance. The plant still continued to evolve carbon dioxide, and when the liquid was distilled it yielded a small quantity of alcohol. The same phenomena manifested themselves when *Mycoderma vini* was similarly treated. But much more marked was the fermentative change when another mould fungus, *Mucor racemosus*, was submerged and grown in a saccharine liquid, as much as 3.5 per cent. (by weight) of alcohol being produced.

Relation between the Weight of the Fungus formed and the Amount of Sugar decomposed.—Pasteur found that when 120 c.c. of wort were impregnated with a minute trace of the spores of *Mucor racemosus*, and the growth resulting from these was kept submerged in the liquid, at the end of the fermentation the plant weighed 0.37 gramme, and the alcohol formed 4 grammes; consequently, an amount of alcohol nearly eleven times as great

¹ Afterwards he expressed a doubt as to whether the plant he had in hand was *Aspergillus glaucus*, or a particular bluish species of *Penicillium* which bore large round spores. This, however, in no way affects the argument.

² Pasteur determined the presence of alcohol in minute traces in a liquid by distilling it from a long-necked retort connected with a Liebig's condenser. When alcohol is present, the first few drops distilled "present the form of little drops or striæ, or, better still, oily tears." In this way the presence of 0.001 part of a volume of alcohol can be detected in a liquid, and with care and practice as little as a tenth part of this quantity. By collecting the first third of the distillate, and redistilling this, even smaller quantities may be detected.

as the weight of the fungus had been produced, and this quantity would require for its formation nearly twenty times the weight of the fungus in sugar. In another similar experiment the weight of *maucedo* formed was 0.25 gramme, and that of the alcohol produced 4.1 grammes, or nearly thirty-three times the weight of the fungus, and this quantity of alcohol would necessitate for its formation the destruction of nearly sixty-six times the weight of the plant in sugar. Obviously, then, when a mould fungus is compelled to live under these forced conditions, it does not, as might be expected, perish for lack of oxygen—in fact, drown—but seems to be able to adapt itself to its new conditions and still maintain its existence. That these conditions are entirely abnormal is shown by the signs of decrepitude which the cells soon begin to show when the plant is compelled to live in this way without a supply of free oxygen. They put on an old, shrivelled, and worn-out appearance; their contours become irregular, and their contents become exceedingly granular. Coincidentally with these appearances the fermentation ceases, and with it the evolution of carbon dioxide. The cells under these conditions are not dead, for, when supplied with a trace of free oxygen, they immediately revive, and fermentation and evolution of gas again commence; when the cells are examined, they are found to have once more put on their pristine appearances of activity. Thus, in order to produce the amounts of alcohol yielded in the experiments just mentioned, it was found necessary to decant the contents of the flask into another one every few days, the slight contact with the air during the decantation being sufficient to revivify the cells.

Anaërobic Growth of Yeast.—In the Jura, wine is manufactured by a peculiar method. The grapes are gathered and placed in large receptacles, which would be entirely closed were it not for an aperture at the top of about four inches in diameter, which is left open for the escape of the carbon dioxide. Each of these receptacles is three parts filled with grapes, some whole, others crushed, and all more or less moistened with the juice of the injured fruits. After a short time the whole mass falls into a state of active fermentation. Obviously, under these circumstances, fermentation with the concomitant formation of a considerable quantity of new yeast must proceed in the absence of free oxygen, for the small quantity of that gas originally contained in the vessel will soon be driven out and replaced by carbon dioxide. In a large vat of fermenting wort the process must likewise be carried on in the entire absence of free oxygen, for the small amount of the gas originally dissolved in the wort will be soon absorbed by the yeast; an access of air to the liquid is afterwards effectually prevented by the layer of carbon dioxide which forms on its surface. Nevertheless, in every such case the yeast-cells not only live, but rapidly multiply and actively exercise their functions for several days; and the immense disparity between the amount of matter which they have actually assimilated, as shown by the weight of the newly-formed yeast, and the amount of sugar decomposed and converted into alcohol, carbon dioxide, and other products, evinces the highly fermentative character which yeast displays when compelled to live under these conditions.

In order to test this question, Pasteur performed the following series of experiments. A large flask holding three litres, the neck of which was drawn out and bent, as in a Pasteur flask, was completely filled with a 5 per cent. solution of sugar-candy in yeast-water,¹ and the open end of the neck immersed in a small dish filled with mercury.

Experiment A.—The liquid contents of the flask were impregnated with the small quantity of yeast obtained by fermenting 15 to 20 c.c. of a solution similar to that contained in the flask, and to which a minute trace of yeast had been added in the body of a small tapped funnel which had been fused on to the short side tube of the flask. In less than twelve days all the sugar completely fermented, and the yeast produced, after being dried, was found to weigh 2.25 grammes, the proportion of yeast to sugar fermented was $\frac{2.25}{150} = \frac{1}{67}$. As such a quantity of liquid would not

absorb more than 16.5 c.c. of oxygen, this is the maximum amount of the free gas which could have been utilised in the fermentation of 150 grammes of sugar. A similar experiment (*C*) was performed, but in this case the sugar solution was almost entirely deprived of air (it contained less than one milligramme of free oxygen) by boiling, and precautions were taken to ensure that the yeast added by the funnel was entirely free from extraneous germs. Simultaneously a parallel experiment (*B*) was made in an ordinary Pasteur flask, double the size of the former flask, the three litres of liquid occupying about one half the flask; consequently a large surface was exposed to the air. The contents of the flask were boiled to ensure sterilisation, and, after cooling, impregnated with a small quantity of yeast from the funnel of the former flask. In the case of the flask (*C*) fermentation commenced on the second day, and on the following days fine and actively budding yeast-cells were observed in the yeast driven out from the end of the tube. The fermentation gradually became more and more languid, and at the end of nineteen days, when the experiment was brought to a close, 4.6 grammes of sugar still remained unfermented. The yeast seemed on examination to be very pure in both cases, and from the sharply-defined appearance of the borders of the cells of that from the first flask, capable of easy revival by aëration; after drying it weighed 1.638 grammes. The proportion of yeast to sugar fermented was, therefore, $\frac{1.638}{145.4} = \frac{1}{89}$. In the flask (*B*), where free exposure to air took

place, the liquid was in active fermentation on the second day, and all the sugar had completely disappeared in eleven days; the yeast from this, after being dried, weighed 1.97 grammes, giving a proportion of yeast to sugar fermented $\frac{1.97}{150} = \frac{1}{76}$.

In a further experiment (*D*) conducted on the same lines as *C*, still greater precautions were taken to ensure the entire absence of air from the

¹ By yeast-water is meant water in which yeast had been boiled, and which, consequently, contained nitrogenous matter and salts extracted from the yeast.

liquid which was impregnated, as before, with a small quantity of yeast from the funnel; instead of using the yeast whilst the contents of the funnel were in an active state of fermentation, at which time the yeast would be in a young and vigorous condition, it was added when fermentation was entirely at an end in the liquid in the funnel. Under these circumstances, fermentation was still going on in the flask at the end of three months, when the experiment was brought to a close. The weight of sugar fermented in this case was only 45 grammes, and the weight of yeast formed was 0.255 gramme, the proportion being $\frac{0.255}{45} = \frac{1}{176}$. The fermentation had evidently proceeded

with great difficulty, and the cells varied much in appearance, some being large and elongated, some very old and granular, others were more transparent, but all might be considered abnormal. In *Experiment A*, where, at the commencement, the liquid was saturated with air, the proportion of sugar to yeast was $\frac{1}{67}$; in *B*, where the liquid had almost been deprived

of air by boiling, but where it was afterwards permitted to have free access to the surface of the liquid, the proportion was $\frac{1}{76}$; in *Experiment C*, where

there was almost an entire absence of air, the proportion was $\frac{1}{89}$; and in the

last (*D*), where air was entirely absent from the liquid and old yeast was employed to start the fermentation, the proportion was $\frac{1}{176}$. From these results Pasteur

concluded that the less free oxygen the yeast has at its command the larger the quantity of sugar which the same amount of yeast is able to ferment when the length of time required for the fermentation is not taken into consideration.

Difference in Fermenting Power of Yeast of Various Ages.—Pasteur points out that it is only young and vigorous yeast which will ferment a solution entirely deprived of free oxygen. If a fermentation be commenced, and, as it progresses, small portions of yeast (as nearly as possible equal in quantity) are successively taken from the yeast which subsides to the bottom of the vessel, and used for starting other fermentations (all of which are, as far as possible, conducted under the same conditions as regards exclusion of air, temperature, &c.), and this continued for some time after the original fermentation has come to an end, it is found with each successive portion of yeast taken, that the first visible signs of fermentation appear later and later the more distant the removal of the portions was, in point of time, from the commencement of the original fermentation.

Microscopical Appearance of Vigorous and of Worn-out Yeast.—If each of these portions of yeast are examined microscopically, those taken at the commencement of the original fermentation will be found to present those appearances which characterise young and vigorous yeast, that is, the cells are somewhat swollen in size, have thin walls, and their contents are plastic

and almost fluid, exhibiting scarcely any signs of granulation. As time progresses, the cells gradually take on the appearance of weakness and exhaustion, the cell-walls become thickened, their protoplasm becomes denser and more and more distinctly granulated. "These progressive changes in the cells," as Pasteur observes, "after they have acquired their normal form and volume, clearly demonstrate the existence of a chemical work of a remarkable intensity, during which their weight increases, although in volume they undergo no sensible change, a fact which we have often characterised as 'the continued life of cells already formed.' We may call this work a process of maturation on the part of the cells, almost the same that we see going on in the case of adult beings in general, who continue to live for a long time, even after they have become incapable of reproduction, and long after their volume has become permanently fixed."

Yeast Cultivated under Conditions of Free Exposure to Air.—The opposite conditions, in which the yeast was allowed to ferment in the presence of more or less free oxygen, were then studied. This was effected, in the first instance, by conducting a fermentation in a flask of 4.7 litres capacity, only 200 c.c. of a 5 per cent. solution of sugar in yeast-water being used. The fermentation was complete in forty-eight hours, and the yeast formed weighed 0.44 gramme; the ratio was therefore $\frac{0.44}{10} = \frac{1}{23}$.

Another experiment was conducted in a flat glass dish, with a layer of liquid that was less than a quarter of an inch in depth, and which consisted of 1.72 grammes of sugar dissolved in 200 c.c. of yeast-water. Fermentation was complete in forty-eight hours, and the yeast weighed 0.127 gramme; the ratio was

$\frac{0.127}{1.04} = \frac{1}{8}$. In another experiment, conducted in a similar receptacle, where

fermentation was only allowed to proceed until sufficient yeast had been formed to permit of its being weighed, the proportion was found to be

$\frac{0.024}{0.098} = \frac{1}{4}$. This was the highest ratio attained, and Pasteur considered that

under these circumstances the yeast more closely approached the manner in which an ordinary mould fungus lives, the eliminated carbon dioxide in this case having been derived from respiration and not from fermentation, which latter process now proceeded very languidly; and that, if it had been possible to keep each yeast-cell entirely surrounded with air, yeast would then have lived just as an ordinary mould fungus does, and vegetated without causing fermentation.

Importance of the Aëration of Yeast and Worts.—These observations and the deductions to be drawn from them are of the greatest importance to the brewer, and their publication threw an entirely new light on the cause of the deterioration which so often takes place in the yeast used in the brewery, and which necessitates its renewal. As pointed out by Pasteur, if it were not for the oxygen the yeast meets with dissolved in the wort, and also that which it seizes upon when manipulated in contact with air (for yeast which

has been deprived for some time of free oxygen absorbs this gas with the greatest avidity when the opportunity is afforded it) it would soon cease to act as a ferment. The enunciation of these principles led to much care being given to the due aëration of wort after it had left the copper; closed or horizontal refrigerators were abandoned in favour of vertical ones, and in more recent times various kinds of apparatus have been contrived for converting the wort into the form of a spray in its passage from the copper to the cooler. The air supplied in this way enables the yeast to preserve its vitality and fermentative faculties.

The Amount of Air which Yeast is capable of absorbing during its Growth.—A number of investigations were made by Pasteur with the object of ascertaining the amount of oxygen which yeast absorbed in carrying out its functions. An endeavour was made to ascertain how much of this element yeast absorbed when caused to live under conditions approaching as nearly as possible those of the ordinary manner of life of a mould fungus. For this purpose a flat-bottomed flask was employed, the neck of which was drawn out and curved as in a Pasteur flask. Into this was introduced 60 c.c. of yeast-water containing in solution 2 per cent. of sugar and the whole impregnated with a minute trace of yeast. The orifice of the fine tube was then sealed up and the flask and its contents kept at a temperature of 25° C. (77° F.) for fifteen hours. The end of the fine tube was then broken off under a jar of mercury, and the gas thus obtained submitted to analysis; and from the amount of oxygen which was found to have been absorbed in this, that of the whole vessel was calculated. In this way it was found that the yeast, which, after being dried, weighed 35 milligrammes, had absorbed 14.5 c.c. of oxygen; consequently, at this rate 1 gramme of yeast would absorb 414 c.c. of oxygen.

Growth of Yeast without a Supply of Free Oxygen.—In *Experiment A*, described on p. 341, 2.25 grammes of yeast were produced in three litres of a saccharine solution under conditions where the liquid could not obtain more than 16.5 c.c. of free oxygen. But if the original cells from which this amount of yeast was derived could not have multiplied without the amount of free oxygen absorbed by the yeast in the previous experiment, that is, 414 c.c. for each gramme of yeast produced, $2.25 \times 414 = 931.5$ c.c. of this gas must have been necessary. The larger portion of the yeast formed in the experiment just mentioned must, therefore, have been an anaërobic growth. Similarly, a mould fungus requires, when grown in its ordinary aërobic condition, a large quantity of oxygen. In the case of a growth of a mould fungus, which had been obtained from a single spore by means of a vacuum flask, the air contained in the flask, when analysed after the lapse of a year, showed that the minute plant, the weight of which, when dried, only amounted to 8 milligrammes, had absorbed no less than 43 c.c. of oxygen that is, in the proportion of 5375 c.c. of this gas for each gramme of plant-substance formed.

Pasteur's Theory of Fermentation.—From a consideration of the deductions to be drawn from these observations and experiments, Pasteur formulated

the following theory of fermentation, which we give in his own words:¹ "Fermentation by yeast, that is to say, by the type of ferments properly so called, is presented to us, in a word, as the direct consequences of the processes of nutrition, assimilation, and life, when these are carried on without the agency of free oxygen. The heat required in the accomplishment of that work must have necessarily been borrowed from the decomposition of the fermentable matter, that is, from the saccharine substance, which, like other unstable substances, liberates heat in undergoing decomposition. Fermentation by means of yeast appears, therefore, to be essentially connected with the property possessed by this minute cellular plant of performing its respiratory functions, somehow or other, with oxygen existing combined with sugar. Its fermentative power—which power must not be confounded with the fermentative activity or the intensity of decomposition in a given time—varies considerably between two limits fixed, by the greatest and least possible access of free oxygen which the plant has in the process of nutrition. If we supply it with sufficient quantity of free oxygen for the necessities of its life, nutrition, and respiratory combustions; in other words, if we cause it to live after the manner of a mould, properly so-called, it ceases to be a ferment; that is, the ratio between the weight of the plant developed and that of the sugar decomposed, which forms its principal food, is similar in amount to that in the case of fungi. On the other hand, if we deprive the yeast of air entirely, or cause it to develop in a saccharine medium deprived of free oxygen, it will multiply just as if air were present, although with less activity, and under these circumstances its fermentative character will be most marked; under these circumstances, moreover, we shall find the greatest disproportion, all other conditions being the same, between the weight of yeast formed and the weight of sugar decomposed. Lastly, if free oxygen occurs in varying quantities, the ferment power of the yeast may pass through all the degrees comprehended between the two extreme limits of which we have just spoken. It seems to us that we could not have a better proof of the direct relation that fermentation bears to life, carried on in the absence of free oxygen, or with a quantity of that gas insufficient for all the acts of nutrition and assimilation."

"Another equally striking proof of the truth of this theory is the fact that the ordinary moulds assume the character of a ferment when compelled to live without air, or with quantities of air too scanty to permit of their organs having around them as much of that element as is necessary for their life as *aërobic* plants. Ferments, therefore, only possess in a higher degree a character which belongs to many common moulds, if not to all, and which they share, probably, more or less with all living cells, namely, the power of living either an *aërobic* or *anaërobic* life, according to the conditions under which they are placed."

Yeast may be grown in an Albuminous Liquid such as Yeast-Water when an Unfermentable Carbohydrate is present.—Pasteur found that when a trace of pure yeast was sown in 150 c.c. of sterilised yeast-water containing $2\frac{1}{2}$ per

cent. of milk-sugar (which is unfermentable by ordinary yeast), the yeast increased, and in three months' time, after being dried, weighed 50 milligrammes. Not the slightest trace of alcohol could be detected in the liquid. In another experiment, yeast which had been grown under precisely similar conditions was found to excite fermentation in sterilised wort readily. These results prove that, under certain circumstances, yeast may live as an ordinary plant, and that fermentation is not an invariable phenomenon of its life, but one which depends on the conditions under which the plant is compelled to live. It is capable of carrying on its existence either as a ferment or not, and even after being grown under conditions where it is unable to exercise its fermentative power, it is able to manifest this when it is again allowed to vegetate under other conditions. It must be noted that yeast when grown in the former manner will only reproduce itself when freely supplied with air. Pasteur concludes that the power of exciting fermentation is not a property enjoyed by cells of a special nature, nor is it a permanent character of any particular structure, but that it is mainly dependent on the conditions of environment under which living cells are placed.

Extension of the Property of exciting Fermentation to every Living Cell.—If, then, as has been stated, the ability to excite fermentation is not a power peculiar to certain cells, there is a strong probability that all living cells, when placed under suitable conditions, will act as ferments. Pasteur felt so certain on this point that one day he said to M. Dumas, "We would lay a wager that if we were to plunge a bunch of grapes into carbon dioxide gas, alcohol and carbon dioxide would immediately be produced as the consequence of a renewed action starting in the interior cells of the grapes, in such a way that these cells would assume the function of yeast-cells." The experiment was made, and on the following day alcohol was detected in the juice of the grapes. No yeast-cells were to be found in the interior of the grapes, although a careful examination was made to see if any were present. Fresh experiments made by immersing grapes, a melon, plums, oranges and rhubarb-leaves gave similar results. Twenty-four plums were placed under a glass cylinder, which was immediately afterwards filled with carbon dioxide, and were allowed to remain for eight days. During this period they evolved a considerable amount of carbon dioxide. They were then crushed and submitted to distillation, when they yielded 6.5 grammes of alcohol, or rather more than 1 per cent. on the weight of the plums. In all these cases there was a liberation of heat, which could be detected by first placing the hand against that portion of the cylinder with which the plums were in contact, and afterwards against another portion where they were not. A somewhat similar series of facts had been demonstrated in 1869 by Lechartier and Bellamy,¹ who found, as had been previously shown by Berard in 1821, that fruits absorb oxygen and liberate almost an equal volume of carbon dioxide. They found that, in addition to the fruits mentioned above, chestnuts, potatoes, grains of wheat, linseed, &c., when any of these were placed in

¹ *Comptes rend. de l'Academie des Sciences*, 1869, 19, 336.

test-tubes and the mouths of the tubes plunged into mercury so as to exclude air, the oxygen of the air in the tube was quickly absorbed and replaced by carbon dioxide. The exhalation of this gas continued for several months, and, when a considerable period had elapsed, notable quantities of alcohol were found. Two pears, which weighed respectively 157 and 125 grammes, were kept under these conditions for seven months; they yielded at the end of that time 2.62 grammes, or about 1.4 per cent. of their weight of alcohol. Beetroots, when kept excluded from the air for a length of time, exhale a strongly acid liquid, and this Pasteur ascribes to the commencement of the lactic and other fermentations.

From these and other facts of a similar nature Pasteur infers, if perhaps only in an indirect way, that the cells of an organism can give rise to several kinds of fermentation. Reasoning from the fact observed by Lechartier and Bellamy, that the proportion of carbon dioxide to alcohol yielded in the case of their experiments with fruits was entirely different to that obtained in the fermentation of wort with beer-yeast, he states: "In the present day it must be borne in mind that the equation of a fermentation varies essentially with the conditions under which that fermentation is accomplished, and that a statement of this equation is a problem no less complicated than that in the case of a living being. Moreover, it is quite erroneous to suppose that the presence of a single one of the products of a fermentation implies the co-existence of a particular ferment. If, for example, we find alcohol among the products of a fermentation, or even alcohol and carbonic acid gas together, this does not prove that the ferment must be an alcoholic ferment, belonging to alcoholic ferments in the strict sense of the term. Nor, again, does the presence of lactic acid necessarily imply the presence of lactic ferment. As a matter of fact, different ferments may give rise to one, or even several identical products. We could not say with certainty, from a purely chemical point of view, that we are dealing, for example, with an alcoholic fermentation, properly so called, and that the yeast of beer must be present in it, if we had not first determined the presence of all the numerous products of that particular fermentation, and that they were present in those proportions characteristic of that fermentation under conditions similar to those under which the fermentation in question had occurred." These extracts show how far-reaching Pasteur's views on the subject of fermentation had become; they were, in fact, not only a denial that there were any special fermentation organisms, but also an affirmation "that every cell may cause every fermentation, alcoholic, lactic, acetic, &c., when deprived of its supply of free oxygen; and the kind of fermentation and of the products formed thereby are merely due to external conditions, the way in which the cells are nourished, and the temperature of the fermentation." "In fact, fermentation is a general phenomenon; it is life without air."

Meyer's Theory of Fermentation.—This philosopher¹ sought to explain the phenomena of fermentation by what he called "intramolecular

¹ *Lehrbuch d. Gährungs-Chemie*, Heidelberg, 1879.

respiration." The cell of every living plant absorbs oxygen, which, by the agency of its protoplasm, is combined with carbon, and again evolved as carbon dioxide. It is, in fact, a process of combustion in which a certain amount of heat is produced, or, in other words, energy liberated which is expended in performing the work necessary to carry on the vital functions of the cell, such as growth, &c. But when a living cell is deprived of the access of free oxygen, it does not cease at once to evolve carbon dioxide; carbon is still consumed in its interior, and the oxygen necessary for effecting this is derived from some of the constituents of the cell. This then is "inner" or "intramolecular respiration." But when oxygen is obtained under these forced conditions, the compounds contained in the cell which furnish this oxygen undergo decomposition and are converted into other bodies, *e.g.*, sugar into alcohol and carbon dioxide. The explosion of gunpowder or nitroglycerine is adduced as a somewhat analogous process; in both these cases combustion ensues, and a large amount of energy is liberated in the entire absence of free oxygen, this element being obtained from a portion of the materials which constitute the explosive. The increase of yeast in the absence of free oxygen is explained in a somewhat similar manner; the ferment organisms under such a condition derive the force necessary for the performance of their vital functions from the oxidation of carbon by intramolecular respiration. Though the growth of yeast is greater when it is supplied with free oxygen, yet it can continue to reproduce itself for a considerable length of time in the absence of this element in the free state. Respiration is then supposed to take place at the expense of the sugar molecule, which is split up in that peculiar way which we characterise as fermentation. Meyer considers that though yeast may cease to grow, it is still able to excite fermentation, because intramolecular respiration can still proceed in yeast though it may have ceased to increase, just as is found to be the case with the cells of the higher plants when they are deprived of the presence of free oxygen.

Though in this latter case the production of alcohol and carbon dioxide may be attributed unquestionably to intramolecular respiration, it is doubtful if it affords a true explanation of the production of these bodies in the ordinary course of fermentation.

Brefeld's Views on Fermentation.—In 1874 and 1875 Brefeld published the results of a number of experiments undertaken with the view of further elucidating the subject of fermentation.¹ His interpretation of the results of these, and the propositions he founded on this interpretation gave rise to considerable discussion and much experimenting at the time, which ultimately threw considerable light on some obscure points in the process. He assumed that the cells of yeast, like all other living cells, must, in order to carry on their vital functions, have a supply of free oxygen. That this was the case with yeast he demonstrated by showing that when placed in an atmosphere entirely devoid of oxygen it very soon ceased to grow, and

¹ *Ber. d. Deutsch. chem. Ges.*, 1874 and 1875.

then a series of characteristic changes commenced in it which ultimately ended in its death. He also proved that it was possible for yeast, when very freely supplied with oxygen, to grow for a time in a saccharine medium without exciting fermentation. From these facts he drew the conclusion that it was only when all the free oxygen in a fermentable liquid had been completely exhausted, and the yeast-cells it contained began to take on the peculiar condition mentioned above, that they commenced to excite fermentation. This they do, according to Brefeld, by secreting an albuminous substance, which, acting on the sugar as an enzyme, causes it to split up into alcohol and carbon dioxide. If the yeast be removed from the saccharine liquid, the secretion of this substance ceases; hence the impossibility of isolating it. Fermentation is, then, according to Brefeld, a pathological process, which only proceeds when the yeast is in a drowning state that ends with its death.

Traube and Pasteur successfully controverted this view by proving experimentally that young and vigorous yeast can increase in a liquid entirely devoid of oxygen. Meyer showed that yeast can simultaneously increase and excite fermentation in a liquid containing an abundance of free oxygen; that fermentation without growth can take place when old cells are immersed in a saccharine liquid which contains no free oxygen; and that young cells, when supplied with a superabundance of this element, can grow without producing fermentation. But, as Meyer points out, between these two extremes there are probably many intermediate stages in which both yeast growth and fermentation can proceed simultaneously. In reality, some of the observations of Brefeld, which were undertaken in support of the theory of Liebig, go to confirm those of Pasteur, such as those where he finds that there is a sort of correlation between the respiration of yeast and its fermentative action, and that the more the former is suppressed the more actively the latter proceeds.

Schutzenberger's Views on the Theory of Fermentation.¹—This observer disputes the validity of Pasteur's doctrine that fermentation is "life without air." With reference to the experiments mentioned on p. 339, where *Mycoderma vini*, when grown immersed in a saccharine medium, was able to act as an alcoholic ferment, Schützenberger is disposed to think that though *Mycoderma*, when grown on the surface of such a liquid, and supplied with an abundance of free oxygen, completely oxidises the sugar to water and carbon dioxide, it may still produce alcohol, which, at the moment of its formation, suffers further decomposition, and hence eludes observation. The volume of the carbon dioxide evolved in these experiments was found to be nearly equal to that of the oxygen which disappeared, and this strongly points to the carbon dioxide being obtained from a carbohydrate, which would coincide with the view that alcohol is primarily formed, and then immediately afterwards broken down to carbon dioxide and water. He also thinks that the phenomena observed by Lœchartier and Bellamy (p. 346)

¹ "On Fermentation," 1886.

may be explained in a similar manner. They prove conclusively that the cells of fruits, under certain circumstances, are capable of transforming sugar into alcohol and carbon dioxide, just as yeast does, only in a much less energetic manner. Schützenberger thinks that under the ordinary circumstances of normal plant life alcohol may be the first-formed product. He states that neither Pasteur nor others have definitely proved that the commencement of this cellular fermentation coincides with the moment when the cells are partially or wholly deprived of free oxygen. Here also alcohol may be the normal production of the cell, which is, under the influence of an abundant supply of oxygen, as in the case of *Mycoderma*, immediately transformed into carbon dioxide and water.

He then draws attention to Pasteur's own statements, that fermentation, when conducted in the entire absence of free oxygen, is very slow ; but when it is carried on under circumstances of free access of oxygen, such as when a large surface of the fermenting liquid is exposed to the air, fermentation proceeds much more rapidly. Pasteur also states that the great activity exhibited at the commencement of a fermentation is due to the air dissolved in the fermenting liquid. These statements seem to be in obvious contradiction to the view that the cause that incites a yeast-cell to ferment is its deprivation of free oxygen. Pasteur, however, explains this apparent contradiction by showing that yeast multiplies with much greater rapidity in the presence of free oxygen, and though the fermentative power of the yeast is lowered, yet the quantity of yeast formed under these circumstances is so much greater, that fermentation, in spite of the lowered fermentative activity of the yeast, proceeds more rapidly. Schützenberger questions the validity of measuring the fermentative power of yeast by the proportion between the weight of the new yeast formed and that of the sugar decomposed, and suggests that a more rational method of measurement would be the quantity of sugar decomposed by the unit weight of yeast in the unit of time. Quoting his own experiments on the absorption of oxygen by yeast, he points out that though yeast absorbs a certain amount of oxygen when it is diffused in water, yet it absorbs much more when placed in an aerated saccharine liquid which also contains nutritive proteid matter. Moreover, he measured the amount of alcohol produced in two fermentations in which all the conditions were identical, with the single exception that in one the fermentation was carried on in the entire absence of free oxygen, in the other the liquid was kept saturated with this gas. In this latter case, strictly in accordance with Pasteur's experiments, the amount of alcohol produced during the same period was sensibly greater, but it was found that during the whole fermentation there had been a continuous absorption of oxygen. If Pasteur's theory be true, under these circumstances the fermentation should have been either stopped or considerably lessened in intensity, because, as there was always a supply of free oxygen at hand, there was no necessity for the yeast to obtain its supply of this gas by decomposing the sugar molecule. According to this, yeast, when placed in a fermentative liquid kept continuously saturated with oxygen—that is, under

conditions where it is able to fully satisfy its respiratory needs with the oxygen—is still able to produce a very active fermentation.

Schützenberger finally concludes that the respiratory and fermentative functions of yeast are two entirely separate and independent powers inherent in the yeast-cell; that they go hand-in-hand; as the one becomes heightened so does the other; and that they do not, as Pasteur assumes, stand in inverse proportion to one another—that is, as the one function is lowered the other is heightened. To demonstrate more fully the correctness of this view, he performed a number of experiments which show that all those cases which tend to lower the vitality of the yeast coincidentally lower both its respiratory and fermentative powers.

Nägeli's Molecular Physical Theory of Fermentation.—This, which differs essentially from all the other theories of fermentation hitherto propounded, was published in 1879.¹ Nägeli considers that the theories so far proposed fail to account for the whole of the observed phenomena, and seeks to establish one which shall more perfectly include them all. By the extension, in a certain sense, of his views on enzymatic action he assumes that the numerous and complicated arrangement of atoms and atomic groups which constitute the protoplasm of a ferment organism are in a state of molecular vibration, each having its own particular rhythm. These vibrations the protoplasm is able to communicate to compounds, which, by diffusion, have actually entered into its substance, or which, dissolved in the liquid, are in close proximity to it. These vibrations acting upon the atoms or atomic groups of the fermentable body cause them to rearrange themselves into new compounds. Since, in this case, we have a number of substances present in the protoplasm, each of which has its own particular rate of vibration and is consequently capable of effecting a different transformation in the fermentable substance, we meet with a great variety of products in fermentation, such as alcohol, glycerol, succinic acid, &c. This also explains why a substance capable of producing fermentation can never be isolated from the living cell: it does not exist. Nägeli considers that there is no necessity for an organism to excrete an enzyme, unless the action has to be performed at a distance from the organism itself. When an organism has to effect a chemical transformation under such circumstances, it then secretes an enzyme, which being able to diffuse itself through a liquid, can travel to and act at a point more or less removed from the organism itself. This we see in the secretion of diastase, &c., by the growing germ of the barleycorn, which, in order to secure its own nourishment, must dissolve tissues situated at a distance from itself. Nägeli considers it very questionable if the organism ever secretes ferments which shall be employed within itself; he thinks that much more powerful energetic means for chemical action are at its disposal in the molecular forces of its own protoplasm. Again, the action of all enzymes is to convert a substance either totally or partially unfitted for the nutrition of an organism into compounds better fitted, as, for example, the conversion of starch into

¹ *Theorie der Gährung*, München.

sugar, albumin into peptone, &c. On the other hand, the substances produced in fermentation are of no further use whatever to the organism ; in fact, they are always prejudicial to it. Thus, yeast in a saccharine medium produces alcohol, which, in sufficient quantity, is absolutely poisonous to it. In enzyme action the chemical compound acted upon is transformed in a simple manner ; each of its molecules suffers identically the same transformation. In fermentation, since the living protoplasm is constituted of many compounds, each of which has its own particular molecular movement, the molecules of the fermentable substance do not each undergo the same change, but are transformed into a variety of bodies, such as alcohol, carbon dioxide, succinic acid, glycerol, &c.; and, moreover, these bodies do not occur in the same proportions in every fermentation ; they vary considerably according as the particular conditions under which each individual fermentation takes place differ. Fermentation is invariably accompanied by the evolution of carbon dioxide, enzyme action never. Enzymes can be replaced by purely chemical agents, such as acids, alkalies, or even heat alone ; fermentation has never been produced by such agents. (Compare, however, Buchner's researches, p. 355.) Such profound differences in the two classes of reactions show that they must be produced by very different agencies. With reference to Pasteur's theory, Nägeli found that the presence of free oxygen favoured fermentation ; consequently, fermentative action could not be caused by the ferment being compelled to derive its supply of oxygen from the sugar molecule. He found that access of free oxygen to the yeast-cells was advantageous, and he assumes that its invigorating action raises the molecular movements of their protoplasm. Nägeli then endeavours to decide whether fermentation takes place inside or outside the yeast-cell. He shows from the results of his own experiments that the yeast-cell, when acting under favourable conditions, decomposes 1.67 times its own weight of sugar and produces 0.85 of its weight of alcohol in an hour. If the fermentation takes place entirely within the cell, there must hourly pass into each of the most vigorous cells 3.34 times its weight of sugar, and out of the cell 1.7 times its weight of alcohol. Though these quantities seem enormous, they were found to be quite within the osmotic capacity of the yeast-cell ; hence no conclusion can be drawn from this as to where fermentation takes place. He then seeks for analogous phenomena in other living cells. No plant-cell is able directly to effect a transformation outside itself or to withdraw anything from an insoluble body so situated. For instance, though yeast-cells are able to withdraw oxygen from the corpuscles of the blood, they only do this indirectly ; they withdraw it by osmosis from the serum in which the corpuscles are bathed, and the corpuscles in turn yield up the gas by osmosis to the serum. Nägeli found that when the thinnest membrane was interposed between the yeast and the fermentable liquid no fermentation took place. He considers that this fact not only indisputably proves that fermentation can take place in the closest proximity to the yeast-cell, but also that it effectually disposes of those theories which assume that fermentation is caused by an enzyme secreted by

the yeast-cell ; such enzyme must necessarily pass through a membrane by osmosis. But this also does not settle the question. The following facts seem to point to the action taking place outside the cell. Nägeli found in 1853 that when certain fruits were immersed, in their entire condition, in grape-juice which had been submitted to the action of the fumes of burning sulphur, fermentation took place in their interior, though no trace of fermentation could be detected in the grape-juice in which they were immersed. But if the fruits, before being treated in this manner, were first carefully deprived of their skins, then no fermentation took place at all. Since yeast-cells or their spores are always to be found on the skins of ripe fruits, he considers that the fermentation in the former case was caused by the vibrations of the protoplasm of the yeast-cells being transmitted through the skin of the fruit into its interior. Nägeli strongly impresses the point that this fermentation is not to be confused with the entirely intra-molecular one discovered by Lechartier and Bellamy, described on p. 346, because the fleshy portion of a fruit when deprived of its skin and adherent yeast-cells, caused no fermentation. Another circumstance which strongly favours the theory that ferment action occurs outside the cell is the formation of acetic ether when yeast and acetic bacteria are present in the same fermentable liquid. The yeast-cells are supposed to produce alcohol in their immediate proximity, and the acetic bacteria similarly acetic acid ; these two bodies unite in their nascent state and produce acetic ether. The prejudicial influence which one energetic ferment exercises upon the nutrition and growth of another ferment seems also to be readily explicable on the assumption that ferment action takes place outside the cell. From experimental data Nägeli calculated that the greatest distance from itself at which an organism could excite fermentation was from one-fortieth to one-thirtieth of a millimetre. He considers that the molecular vibrations of the protoplasm of a ferment organism are transmitted in the same way as those of sound, light, or heat. The vibrations of one molecule set up vibrations in another, and these again in others, and so on. The vibrations of the protoplasm are first communicated to the sugar molecules which have penetrated into the interior of the yeast-cell by osmosis ; these molecules transmit their vibrations to the sugar molecules contained in the membrane surrounding the yeast-cell, which is saturated with the saccharine solution, and these, in turn, to the sugar molecules dissolved in the liquid outside the cell. Finally, Nägeli concludes that the cause of fermentation is the living protoplasm of the ferment organism, and therefore resides in its interior, but that it is able to extend its sphere of action to a distance of at least one-fiftieth of a millimetre beyond the investing membrane of such organism. The actual fermentation action is supposed to take place to a small extent inside the cell, but to a much greater extent outside.

Nägeli considers that this is true only with reference to the principal products of fermentation, *i.e.*, alcohol and carbon dioxide ; the subsidiary products, glycerol, succinic acid, &c., are most probably entirely formed within the cell.

He thinks that the same theory may be extended to all other fermentations—lactic, butyric, putrefactive, &c. The acetic fermentation, in which alcohol becomes oxidised to acetic acid, may be explained as follows: The molecular vibrations of the protoplasm of the acetic bacterium are communicated to the alcohol and oxygen molecules absorbed by the organism, and by them transmitted to the alcohol and oxygen molecules outside the cell. When the disturbance of the equilibrium of these molecules has reached a certain degree of intensity, a molecular transformation, *i.e.*, a chemical change, ensues. A part of the process takes place in the interior of the cell, but the greater part outside.

He considers that the action of a ferment may be divided into two stages: in the first of these the equilibrium of the molecules is disturbed, and to effect this a small amount of energy is expended by the yeast-cell; in the second stage, by virtue of the new attractions and repulsions brought into play amongst the molecules, new phases of equilibrium become established among them, which result in the formation of new compounds, and this process is accompanied by the liberation of energy, which appears as heat.

Hoppe-Seyler's Theory of Fermentation.—This physiologist¹ considers that fermentation is caused by an enzyme secreted by the yeast, and that the division of ferments into organised and unorganised is a fallacy.

If the whole organism is to be included under the denomination "ferment," then no division is made between it as a whole and its several constituents, nor between its whole life and the individual processes of its life. The idea of an organised ferment, in Pasteur's sense, might be applied to every organism, even to man himself; the ferment (enzyme) which converts glucose into alcohol and carbon dioxide is identified with the whole organism of the yeast-cell, and the production of glycerol and succinic acid are assumed to be connected with the same process. But the yeast-cell produces fat, cellulose, invertase, &c., and Hoppe-Seyler considers that to assume that one series of vital processes is inseparably connected with the whole life of the cell, and that another series is not, is quite an arbitrary assumption.

It has been hitherto supposed that oxidation in the living organism was purely a process of addition, similar to that which is effected by ozone or potassium permanganate. It was thought that oxygen combined directly with organic substances and converted them into compounds becoming progressively richer and richer in oxygen, until the final stages of water and carbon dioxide were reached. This view is, however, contradicted by recent observations, and it seems much more probable that the transformation process depends essentially upon the action of water, which, though itself a comparatively inert substance, yet, when under certain conditions co-operating with those organic bodies which we call enzymes, can then cause profound changes. All organisms contain and produce enzymes, and the higher organisms produce a large number of different varieties. The organisms which are concerned with fermentation are, according to Hoppe-Seyler, able

¹ *Physiolog. Chemie.*

to produce and to be the bearers of ferments. These are just as inseparable from the organism as the contractile substance is from the muscle, or the protoplasm from the organism itself; with the death of the organism the ferments cease to act.

Water, according to Hoppe-Seyler, is essentially necessary for fermentation. In ferment action, according to his theory, the oxygen atoms split up from the hydrogen atoms and attach themselves to the carbon atoms; the liberated hydrogen, being in the nascent state, converts any oxygen which may be present into active oxygen.

In the alcoholic fermentation the sugar molecule is split up by the ferment into two atomic groups, and two molecules of water are split up into two hydrogen atoms (H_2) and one molecule of hydrogen peroxide, H_2O_2 . The oxygen of the peroxide exercises an oxidising action on the two molecular groups into which the sugar is split up, the hydrogen simultaneously exercising a reducing action. Through this combined action two molecules of carbonic acid, $CO(OH)_2$, and two molecules of alcohol are yielded, thus:—

The carbonic acid then breaks up into water and carbon dioxide. The production of glycerol, succinic acid, and the higher alcohols (fusel oil) are not considered to be connected with this process, which is essentially concerned in the decomposition of the sugar.

Buchner's Researches on Alcoholic Fermentation.—In 1897, E. Buchner proved¹ that the liquid forming the cell contents of yeast-cells is able to effect alcoholic fermentation when added to a solution of a fermentable sugar, and the mixture kept under aseptic conditions. One kilogramme of fresh brewing-yeast freed as much as possible from adherent water by being submitted to a pressure of about 350 lb. to the square inch, was ground with an equal weight of quartz sand and 250 grammes of kieselguhr until the mass became soft and plastic. With it 100 cc. of water were intimately mixed, the whole wrapped in a cloth, and submitted to a pressure of from 400 to 500 atmospheres (about three to four tons to the square inch) in a hydraulic press; about 300 cc. of yeast-extract were obtained in this way. The cake was removed from the cloth, powdered, sifted, 100 cc. of water mixed with it, and again similarly submitted to hydraulic pressure; in this way another 150 cc. of yeast-extract were obtained. The secret of the operation appears to lie in the complete rupture of the yeast-cells, which can only be accomplished by long grinding. The expressed yeast-extract thus obtained was a clear, slightly opalescent, yellow liquid, of a specific gravity of 1.042, having a pleasant yeast-like odour. On being boiled, coagulation ensued to such an extent that it became a semi-solid mass. The extract contained about 10 per cent. of solid matter in solution.

When equal volumes of the expressed yeast-liquid and of a concentrated

¹ *Berichte d. Deutsch. chem. Ges.*, 1897, **30**, 117.

solution of either glucose, fructose, cane sugar, or maltose were mixed, a regular evolution of carbon dioxide commenced in from fifteen minutes to an hour, which lasted several days. A solution of this kind, in which fermentation had been proceeding for several days, gradually became turbid from the coagulation of proteid matters when kept in an ice-chamber, but no trace of micro-organisms could be detected in it. The addition of chloroform did not arrest fermentation, and a portion of the extract, which had been passed through a Berkefeld filter (which would certainly retain any micro-organisms the size of a yeast-cell), when mixed with a sterilised solution of cane sugar, fell into a state of fermentation in about a day's time, at the temperature of an ice-chamber. When a parchment-paper tube filled with the yeast-extract was suspended in a 37 per cent. solution of cane sugar, minute bubbles of gas made their appearance on its surface in a few hours, whilst the contents of the tube fell into an active state of fermentation owing to the sugar which had diffused into the tube. The fermentative power of the extract gradually disappears; thus, a quantity kept in an ice-chamber for five days was found to be unable to act upon a cane sugar solution; but, on the contrary, when a quantity of fresh extract was added to such a solution, and the mixture placed in an ice-chamber, fermentation continued for a fortnight.

In an attempt to ascertain the nature of the substance present in the yeast-extract which caused fermentation, it was found that when the liquid was heated for an hour to from 40° to 50°C. , the filtrate from the coagulum in one experiment excited a feeble fermentation in a solution of cane sugar, but in another experiment it proved inactive. Evidently the active substance was coagulated at this comparatively low temperature.

Zymase.—Buchner concluded from these experiments that the active agent in the expressed cell juice was an enzyme and for this he proposed the name "zymase." The principal results of Buchner and his colleagues have been collectively published in a volume.¹ The original papers are for the most part published in the *Berichte* of the German Chemical Society. The principal facts elucidated are summarised in what follows:

The activity of expressed yeast juice is not affected by the presence or absence of air, but it loses its activity on keeping. The fermentative action of expressed yeast extract has been proved not to be caused by minute fragments of yeast-protoplasm suspended in the liquid. The instability of the extract is not due to the action of atmospheric oxygen, but probably to that of a proteolytic enzyme; and this latter hypothesis also explains the preservative influence of a concentrated solution of cane sugar, which, it is considered, would destroy the activity of the proteolytic enzyme. Antiseptics, such as chloroform, benzene, and sodium arsenite, do not destroy the activity of the yeast-extract; but, as they possess a powerful inhibitory influence on the growth of living yeast-cells, it may be presumed that their influence on portions of protoplasm deprived of a protective cell-wall would be still greater. The yeast-extract may be evaporated to dryness by exposing it in thin layers

¹ *Die Zymasegärung*, 1903.

to a temperature of from 86° to 95° F. in a partial vacuum. When the solid matter obtained from the extract in this way is again dissolved in water and added to a 75 per cent. solution of cane sugar, fermentation commences in from six to ten hours, and lasts for several days, the dry residue retaining its activity for twenty days. It was also found possible to isolate an active substance from the extract by precipitation with alcohol.

As a further proof of the validity of the theory, that the activity of the expressed juice is due to the presence of an enzyme, it has been found that yeast which had been dried at 98.6° F., and afterwards exposed for six hours to a temperature of 212° F., by which the yeast-cells were killed, when added to a sterilised solution of cane sugar, was able to induce a vigorous fermentation. If, however, yeast dried in a similar manner was exposed to a temperature of 284° to 293° F., the active principle, and with it the fermentative power of the yeast, was destroyed.

The preparation of a yeast-extract capable of exciting alcoholic fermentation presents numerous difficulties, but soon after the publication of Buchner's results it was successfully carried out by Delbrück in Berlin and by Reynolds Green in this country.

In support of the view that the activity of yeast-juice is not due to the presence of micro-organisms Buchner and Rapp have shown that it may be filtered through a Chamberland filter without losing its activity; and that it becomes inactive when kept for a few days. Hans Buchner found that the addition of sufficient cane sugar or dextrose to wort until a 44 per cent. solution was formed completely arrested fermentation by living yeast-cells. Fifty per cent. solutions of these sugars may, however, be fermented with zymase. Hydrocyanic acid completely checks the activity of zymase, but this may be restored by subsequently passing a current of air through the solution or by addition of hydrogen peroxide. At ordinary temperatures the fermentative power of zymase in cane sugar solutions diminishes rapidly after the first twenty-four hours and falls to zero in three to four days. The fermentative power of zymase is several hundred times less than that of living yeast. At higher temperatures the action of zymase is at first intensified, but subsequently diminishes more rapidly than at low temperatures. One to two per cent. of arsenious oxide dissolved in potassium carbonate may be added to zymase without sensibly affecting the fermentative activity. When yeast juice is kept at 32° F. very little change occurs in twenty four hours, but at 44 – 46° F. the activity diminishes to less than half in the same period. The activity of zymase is not influenced by yeast-cells, nor is the activity of yeast juice which has become inactive by keeping, restored by the action of living yeast.

Permanent Yeast.—Buchner has patented the process of obtaining the cell contents of micro-organisms by submitting them after mixing with sand to pressure (German Patent, 99,508). He has also patented a process of preparing dried yeast containing zymase (German Patent, 97,240). The preparation is denoted "Dauerhefe" (permanent yeast). The washed yeast is

dried in thin layers at a low temperature and subsequently heated at 122–212° F. until the yeast is killed. The product may then be ground; an addition of 5 to 10 per cent. of it to flour suffices for raising dough.

Abeles in 1898 brought forward evidence in support of the view that the fermentative power of expressed yeast juice was due to the presence of particles of living protoplasm. This evidence was subsequently refuted by Buchner and Rapp (see also Albert's Experiments p. 359).

Experiments of Macfadyen, Morris, and Rowland.¹—An important paper was published by these observers, of which the following is a summary:—The top fermentation yeast of the English breweries yields, on suitable treatment, an expressed extract which possesses the transient property of converting sugar into alcohol and carbon dioxide. The quantity of gas evolved by an active extract is equal to, or greater than, that obtained by Buchner. The extracts prepared were subject to considerable auto-fermentation, the yield of gas from which exceeded, in some cases, that obtained by mixing the same samples with cane sugar solution. A moderate dilution (1 : 2) with water, or with a 0.75 per cent. solution of sodium chloride, practically destroyed all the fermentative properties of the extracts. Only in the case of a very active extract was the ratio of alcohol to carbon dioxide comparable with that of ordinary alcoholic fermentation. When the expressed extract was allowed to act on sugar (cane sugar or dextrose), the quantity of sugar destroyed was considerably greater than that required for the production of the carbon dioxide and alcohol obtained. Macfadyen, Morris, and Rowland believed that their results indicated that fermentation by means of expressed yeast extract is a phenomenon of the vital activity of the yeast protoplasm, and is not to be ascribed to an enzyme. They centrifuged the yeast before grinding, and they devised a machine for breaking up the cells with sand without manual labour. The length of time between collecting the yeast and grinding it was found to have a considerable influence on the yield and activity of the extract obtained; the activity appeared to reach a maximum on the third or fourth day. The fermentative power rapidly decreased on keeping the extract, owing to the energetic action of the proteolytic enzyme. Lower concentrations (10 per cent.) of sugar solutions yielded more favourable results than stronger ones; cane sugar was fermented somewhat better than other sugars.

Buchner has replied to the above criticisms of his results, and at the present time the existence of zymase is practically universally admitted.

Buchner and Rapp state that expressed yeast-extract evaporated *in vacuo* and carefully dried over sulphuric acid has been kept for twelve months without appreciable loss of fermentative power. The addition of certain salts—ammonium chloride, calcium chloride, sodium, ammonium, or magnesium sulphate, borax, and, to a less extent, barium chloride (probably on account of its high molecular weight)—has a considerable retarding influence on the activity of the enzyme. The presence of electrolytes seems to modify the

¹ *Berichte d. Deutsch. chem. Ges.*, 1900, **33**, 2764.

colloidal condition. Wroblewski's observation that the addition of nitrites to the juice causes an evolution of nitrogen by inter-action with the amino-compounds has been confirmed. The amount of the by-products (glycerol and succinic acid) formed in fermentations by zymase has been shown by Buchner and Rapp to be considerably less than in the case of ordinary fermentation by living yeast, but although the zymase fermentation was conducted in presence of potassium metarsenite the development of yeast-cells was not entirely prevented.

Albert's Experiments.—One of the most conclusive pieces of evidence in support of the enzyme theory of alcoholic fermentation is that brought forward by R. Albert¹ who describes a method whereby yeast is killed, whilst at the same time its fermentative properties are preserved. Washed and pressed yeast (250 grammes) is squeezed through a hair sieve into a mixture of absolute alcohol (3 litres) and ether (1 litre). After four to five minutes the liquid is decanted, and the yeast washed with ether (500 c.c.) and dried in thin layers on filter paper. The yellowish white powder thus obtained contains no living cells. It is shown that preparations of this powder may be five times as active as expressed juice. Since fermentation occurs within the cells, while the latter remain unbroken water fails to extract any zymase from them; an active extract may, however, be obtained by trituration with sand, at first dry and subsequently with the addition of water. Albert in conjunction with Buchner and Rapp subsequently recommended acetone instead of alcohol and ether for killing the yeast; the product so obtained is denoted "*Acetone Dauerhefe*" (see p. 358).

Formation of Zymase in Yeast.—Albert has shown that the quantity of zymase in yeast may be increased by applying Hayduck's method of regeneration, *i.e.*, cultivation at 60° F. in a well aerated sugar solution containing very little nitrogenous compounds. He found that less zymase was present in the yeast at the highest stage of fermentation than was found some time after this point had been passed. Delbrück and others have proved that the zymase content of living yeast varies greatly when the yeast is kept in the pressed state, increasing on storage at low temperatures and decreasing at higher temperatures. Buchner and Spitta state that the only objection to this conclusion lies in the liability of the quantity of zymase in the yeast to change in the course of the determination of its fermentative power. This they have overcome by killing the yeast cells at any desired stage by treatment with alcohol and ether or acetone as above described. In this way they have studied the quantity of zymase secreted by the yeast at various stages in the course of Hayduck's process of regeneration. In confirmation of Albert's conclusions they found that yeast withdrawn and killed at the highest stage of fermentation contains little zymase, so that although the production of zymase must here be at a maximum, it is apparently destroyed as it is formed. By keeping yeast withdrawn at this stage in the pressed condition at a low temperature for 2–3½ hours before killing it, the quantity of

¹ *Berichte d. Deutsch. chem. Ges.*, 1900, **33**, 3775.

zymase was very considerably increased. On the other hand, in the case of yeast taken before regeneration or after the highest stage of fermentation was passed, similar storage at low temperatures had no such effect. "Regenerated" yeast is therefore not necessarily that which contains much zymase ready formed but rather that which is in such a condition as to be able to produce this enzyme rapidly.

Chemical Mechanism of Alcoholic Fermentation.—Buchner and Meissenheimer¹ draw attention to the fact that in cell-free fermentation it is never possible to obtain the full amount of alcohol corresponding with the sugar which has disappeared, lactic acid and acetic acid being invariably produced. The formation of these acids takes place under conditions which preclude the activity of bacteria or the presence of air. Numerous experiments were carried out in which expressed yeast juice (to which 1 per cent. of toluene had been added) was allowed to act on sugars. From the results the authors are led to conclude that lactic acid is an intermediate product in the alcoholic fermentation of sugars, and they suggest a modification of von Baeyer's hypothesis,² thus:

Further investigations of Buchner and Meisenheimer³ have confirmed their conclusions that lactic acid is an intermediate product in alcoholic fermentation. They conclude moreover that the fermentative action is the result of an equilibrium of the action of two enzymes, one, to which they propose to confine the name "yeast-zymase," converting sugar into lactic acid, and the other, to which they apply the name "lactacidase," converting lactic acid into alcohol and carbon dioxide.

Harden's Researches on Zymase and Alcoholic Fermentation.—A valuable *résumé* of the researches of this chemist will be found in the *Journal of the Institute of Brewing* (1905, 11, 2-15). He showed in 1903 that the addition of the blood serum of the rabbit, pig or horse to expressed yeast juice restricts the activity of the proteolytic enzyme and increases the evolution of carbon dioxide as much as 80-90 per cent. when sugar is added to

¹ *Berichte Deutsch. chem. Ges.*, 1904, **37**, 417

² *Ber. Deutsch. chem. Ges.*, 1870, **3**, 73.

³ *Ibid.*, 1905, **38**, 620.

the juice. He has since found that when boiled yeast juice is filtered and added to fresh juice, a very considerable increase in the fermentative power is produced, which increase is greater, the larger the quantity of boiled extract added. The boiled extract has also a somewhat irregular retarding effect upon the proteolytic enzyme, but apparently this effect is independent of that on the fermentative enzyme. The addition of varying volumes of boiled juice affects the two enzymes in totally different manners; in the case of the zymase the maximum result is reached slowly and in that of the proteolytic enzyme the maximum effect is very quickly reached and then remains constant. The nature of the substance which stimulates the alcoholic fermentation is not known, but it is precipitated by alcohol and is unaffected by boiling, but is not produced thereby. It is present in both fresh and old juice and will pass through a dialyser. Experiments on the dialysis of fresh yeast juice appear to indicate that zymase by itself is incapable of effecting alcoholic fermentation, and that the presence of this stimulating substance is necessary before the activity of the zymase is manifested. Membranes of gelatin were prepared in the pores of a Chamberland filter and the fresh yeast juice was forced through this under the pressure of fifty atmospheres. It was then found that neither the filtrate nor the residue on the filter was capable of setting up a sensible fermentation in sugar solutions, but that when the residue and filtrate were mixed together a fermentation was produced comparable in magnitude with that set up by the original juice.

Experiments of Adrian Brown on Fermentation.—Though the theory of fermentation propounded by Pasteur is perhaps the one which has met with the widest acceptance, yet in later times numerous observations have been made which tend to throw considerable doubt upon its validity. We have seen how both Schützenberger and Nägeli found that fermentation by yeast was aided rather than hindered by the presence of oxygen. Blankenhorn observed the same thing in the process of wine-making; at the end of the fermentation of the grape-juice a certain amount of sugar is usually left unfermented, and if at this stage the young wine be aerated, fermentation commences anew, and the last portions of sugar disappear. More striking still are the experiments of Adrian Brown, performed with a view to the further elucidation of the phenomena of fermentation. In these, following the example of Pedersen, Hansen, and others, he employed a method of counting the yeast-cells formed at the various stages of the process by means of an instrument which, as it was originally invented to count the red corpuscles of the blood, is called a “*hæmacytometer*.”

The Hæmacytometer and the Method of Using it.—This instrument consists of an ordinary glass slip, to which is cemented a cover glass of a definite thickness, say one-tenth of a millimetre, in which a circular aperture has been cut. This forms a shallow trough one-tenth of a millimetre in depth; consequently, when a drop of liquid is placed in the trough and covered with a thin cover-glass, it is evident that we have a layer of liquid one-tenth

millimetre in thickness. On the bottom of the trough a series of squares are engraved, the sides of which are one-twentieth of a millimetre in length ; consequently, the area of each square will be $\frac{1}{20} \times \frac{1}{20} = \frac{1}{400}$ of a square millimetre, and the amount of liquid represented by one of these squares is $\frac{1}{400} \times \frac{1}{10} = \frac{1}{4000}$ of a cubic millimetre. If a drop of liquid containing yeast-cells is placed in the trough, taking care that the drop is of such a size as to nearly fill the trough, but not so large as to exude when the cover-glass is put in its place, and the whole allowed to remain at rest for a short time, the yeast-cells will settle to the bottom of the trough. The slide is then placed under the microscope, and the number of yeast-cells counted gives the average number of cells contained in each $\frac{1}{4000}$ of a cubic millimetre of the liquid. For instance, if, on counting 64 squares, 168 cells are found, then this number divided by 64 will give 2.625 as the number of cells per $\frac{1}{4000}$ cubic millimetre. Whatever the volume per square of the instrument may be, this is taken as the standard volume ; and as all calculations are made to this one volume some little latitude in its size is permissible. Before placing the liquid in the instrument, it is necessary to agitate it for some time, so that the yeast-cells may be evenly distributed and an average sample obtained ; as also to see that the yeast-cells are not too numerous to be conveniently counted ; this may always be secured by sufficiently diluting the liquid and making a subsequent allowance for the amount of dilution. The liquid used should have a specific gravity so high that the yeast-cells do not sink in it too rapidly, and yet not so high as to cause them to take an inconvenient length of time in subsiding. Wort of the strength usually used in brewing operations offers no difficulties in either of these directions. In order to prevent the yeast-cells from clinging together, and also for the purpose of stopping the growth of the cells, Hansen recommends the addition of one or two volumes of 10 per cent. sulphuric acid.

The Amount of Increase of the Yeast-cells in a Fermenting Liquid is Determined by the Volume of the Liquid.—Adrian Brown¹ found that when equal quantities of the same fermentable liquid were seeded, the one portion with 0.93 yeast-cell per standard volume,² and the other with 7.44, at the conclusion of the fermentation the number of yeast-cells found in each of the two solutions was 25.14 and 27.08 respectively—that is, practically the same number. From this and other similar experiments it was conclusively shown that the number of cells found at the end of a fermentation was little influenced by the number added at its commencement, provided they did not exceed a certain number ; in fact, that the number of yeast-cells found at the end of a fermentation was dependent, to a great extent, on the volume of the liquid ; in other words, that with a certain volume of liquid there was a certain maximum beyond which no further increase of yeast-cells appeared possible. By adding more yeast-cells at the commencement of a fermentation than was

¹ *Journ. Chem. Soc. Trans.*, 1892, **61**, 369.

² The standard volume used in all these experiments was $\frac{1}{4000}$ of a cubic millimetre.

the maximum for the particular volume of liquid being used, the further increase of yeast-cells was almost entirely suppressed; and when two or three times this maximum number was added, then no further increase, as evinced by signs of budding, was visible. By working in this way, it was possible to eliminate from a fermentation all the influences due to the multiplication of the yeast.

In two parallel experiments conducted under conditions where this large excess of yeast was employed, air was caused to bubble continuously through the liquid in the one case, whilst the other was conducted in the ordinary manner. It was invariably found in those experiments which were liberally supplied with air that the most sugar had disappeared in a given interval of time. In order to ascertain if this increase of fermenting power was caused by the agitation of the liquid by the air bubbling through it, parallel experiments were made in which either carbon dioxide or hydrogen was passed through the liquid in a similar manner. Fermentation, however, always took place with greater rapidity in those cases where the liquid was liberally supplied with air. These results, therefore, stand in direct contradiction with the hypothesis of Pasteur, that under the influence of free aëration the fermentative activity of yeast is diminished.

In a subsequent paper,¹ Brown brought forward further arguments and fresh experiments in support of his contention. The discussion is confined to fermentation produced by yeast alone, and no reference is made to those experiments with mould fungi, bacteria, &c., by which Pasteur sought to extend to every living cell the property of being able to excite fermentation, since these latter could but strengthen the theory by analogy after it had been conclusively proved to be true with regard to yeast. Brown considers that Pasteur's experiments may be divided into two classes, the first of which suggests the probability of the correctness of his theory, the second those by which he attempts to prove its truth. To the first class belongs the experiment on p. 343, where a large quantity of oxygen was absorbed by yeast when grown in a thin layer of fermentable liquid freely exposed to the air; this, however, merely proves that yeast is able to grow under aërobic conditions, and gives no information concerning its fermentative functions. So the experiments on pp. 341 and 342, which show that yeast can grow and carry on a brisk fermentation in a liquid when entirely cut off from access of air, only demonstrate the fact that yeast can also live under anaërobic conditions, and that yeast, when existing under the latter conditions, can cause fermentation.

To the second class belong those experiments which tend to show that the more yeast is caused to act under anaërobic conditions, the greater becomes its fermentative power; and on these conclusions is based the theory that fermentation is "life without air." But as Brown points out, if Pasteur's experiments are to prove his case conclusively, "two points are

¹ *Journ. Chem. Soc. Trans.*, 1894, 65, 911.

essential—first, that the ratios of the weight of the yeast formed to sugar fermented should be numerical expressions of the fermentative powers of the yeast; and, secondly, that these ratios should be comparable with each other. For by comparisons between these ratios Pasteur determined the relative fermentative powers of the yeast-cells under varying conditions of experiment, and established his theory upon the conclusions thus arrived at. If, then, the ratios of yeast to fermented sugar do not express the true fermentative powers of the yeasts, or if these ratios are not comparable, Pasteur's deductions fail, and his theory remains without experimental proof." According as fermentations were carried out under different conditions with reference to air-supply, the proportions of yeast formed to sugar fermented varied from 1 : 176 to 1 : 4; that is, the greater the supply of air the less the yeast acted as a ferment, and the nearer it approached the manner of living of an ordinary fungus. But, as Brown remarks, the ratios given are assumed to express fermentative powers that are comparable with each other; and in order to do this correctly, the numbers given must either express the total or some known fraction of the fermentative power of the yeast; though, undoubtedly, Pasteur assumes that these numbers expressed the total fermentative power which the yeast was capable of exerting. In the experiment in flask C (p. 341), as sugar was present in the solution when the experiment was terminated, the yeast had been able to exercise its fermentative powers during its whole existence, and here, perhaps, its total power was represented as nearly as possible, though a very languid fermentation was still proceeding. In the experiment in flask B, as the whole of the sugar was fermented, the fermentation must necessarily have come to an end, and no evidence is afforded that in this case the whole of the fermentative power of the yeast was obtained, since, had the yeast had more sugar at its command, it would undoubtedly have still continued to exercise its fermentative functions. Still, Pasteur assumes that the ratios obtained in these two instances were comparable. This would be true if the ratio of yeast produced to sugar fermented was a constant one, but such is by no means the case, as the following experiments show: Five fermentations were conducted, each with 100 c.c. of sterilised yeast-water, to which weights of cane sugar varying from 2.5 to 30 grammes were added, each being impregnated with a trace of a pure culture of top fermentation yeast. The results are appended in the following table:

No. of Experiment.	Time of Fermentation.	Grammes of Sugar in Solution.	Grammes of Sugar Fermented.	Weight of Yeast Formed, in Grammes.	No. of Cells per Standard Vol.	Ratio of Yeast to Sugar Fermented.
1	5 days	2.5	2.5	0.1240	8.51	1 : 20.2
2	5 days	5.0	5.0	0.1550	9.94	1 : 32.3
3	7 days	10.0	10.0	0.1775	10.44	1 : 56.3
4	12 days	20.0	20.0	0.1400	11.17	1 : 142.9
5	20 days	30.0	25.17	0.1380	12.26	1 : 182.4

These results show how little the weight of yeast formed is influenced by the strength of the solution. The yeast increases in weight in solutions up to 10 per cent. strength, after which there is a diminution as the solution becomes stronger.¹ These differences in the weights are very slight, and distinctly prove that the weight of the yeast formed bears no definite relation to the weight of the sugar fermented. According to Pasteur's views these ratios represent the fermentative powers of the yeasts, and should therefore be comparable with one another. If this were so, then the yeast in experiment 4 had about four and a half times the fermentative power of the yeast in experiment 2, and in both these cases the sugar was entirely fermented. The only difference in the conditions of the two experiments was the amount of sugar present, and probably the reason why the yeast exhibited this increased fermenting power was because it had more sugar to act upon. Nothing in either case shows that the yeasts had exerted more than an unknown fraction of their fermenting power. In experiment 5 sugar was still present at the termination of the fermentation; consequently the yeast here decomposed as much sugar as it was capable of doing. In this case it is notable that the ratio of yeast formed to sugar decomposed was 1:182, a lower result than the lowest obtained by Pasteur when working under the most perfect anaërobic conditions; yet air had access to the fermenting liquid all the time. Brown further concludes that though there are many other factors concerned in fermentation, the amount of sugar present is the principal one influencing the ratio of the weight of yeast formed to that of the sugar fermented, and not the relative condition of aëration or non-aëration. The following experiments show clearly that the true ratio is not given by experiments of such a nature as that of B (p. 341). Three flasks numbered 1, 2, and 3, each containing 200 c.c. of a 5 per cent. solution of cane sugar in yeast-water, had their mouths closed with cotton-wool plugs, and were sterilised. One of these, No. 1, was so arranged that when the fermentation in it was ended the solution could be partially withdrawn and replaced by fresh. Each was impregnated with a trace of a pure culture of top fermentation yeast, and kept at a temperature of 26° C. (79° F.). In four days fermentation had ceased, and in flask 2 the ratio of yeast formed to sugar fermented was found to be 1:29. The same day 120 c.c. of the fermented liquid were removed from flask 1, and 50 c.c.² of a solution containing 10 grammes of sugar were passed into it. A rapid fermentation ensued, which ceased in three days, when a further portion of the fermented liquid was again removed, and replaced by another 50 c.c. of a solution containing 10 grammes of sugar. Fermentation was over in another six days, and the yeast-cells were then counted and weighed. The yeast in flask 3, which had remained at rest the whole time, was similarly treated. The results of the three experiments are appended:

¹ The yeast-cells still continue to increase in number, but become smaller in size.

² The volume of this solution was kept small in order to prevent any multiplication of the yeast-cells, as this would have necessarily vitiated the result of the experiment.

No. of Experiment.	Duration of Experiment.	No. of Cells Found per Standard Vol.	Total Weight of Yeast Found, in Grammes.	Total Sugar Fermented in Grammes.	Ratio of Yeast, to Sugar Fermented.
1	13 days	9.38	0.2990	30	1 : 100.4
2	8 days	9.02	0.3435	10	1 : 29.1
3	13 days	9.40	0.2435	10	1 : 41.0

Though the number of yeast-cells in each experiment was practically the same, three times as much sugar was fermented in experiment 1 as in 2 and 3. The weight of the yeast formed in the three experiments somewhat differs, and has decreased in experiments 1 and 3. In the latter the decrease is probably owing to the fact that it had remained in an exhausted solution for some time, and it is known that yeast does lose weight under these conditions. The yeast in experiment 1 had actually been employed in fermenting all the time, and the fact that it had lost weight distinctly proves that the fermentative functions of the yeast-cell are not in any way connected with its growth and development. These experiments, then, distinctly show that the ratios of yeast formed to sugar fermented do not express the true fermentative powers of the yeast, or any known fraction of that power, when the yeast has only a limited amount of sugar at its command, and such ratios are therefore not comparable. With reference to Pasteur's experiment on p. 343, where by growing yeast under conditions of very free aëration, and where the ratio of yeast formed to sugar fermented was 1 : 4, Brown objects that here the element of time was introduced; fermentation was arrested when there was sufficient yeast formed to be weighable, its action was limited in point of time, and there was nothing to show that if the experiment had been continued the yeast would not have gone on fermenting. Hence, according to Pasteur's own views, the ratio thus obtained does not represent the fermentative power of the yeast, but its fermentative energy.

But there is still a further objection to this experiment: Pasteur employed cane sugar. It has been distinctly shown by J. O'Sullivan¹ that the fermentation of cane sugar is invariably preceded by its inversion, and Brown proves, by the following experiments, that during the time that Pasteur allowed his experiment to proceed, the yeast was really occupied in inverting the sugar. Fermentations were started in six flasks, with, in each case, a trace of yeast. Three of the flasks contained a 10 per cent. solution of cane sugar in yeast water, the remaining three a similar solution of glucose. In forty-two hours, when sufficient yeast had formed to be weighed, the experiment was stopped in one flask of each series, and the ratio of yeast formed to sugar fermented determined. The same operation was performed in two other flasks at the end of sixty-six hours, and with the remaining two at the end of ninety hours. The results are appended :

¹ *Journ. Chem. Soc. Trans.*, 1892, 61, 593.

Cane Sugar Experiments.				Glucose Experiments.		
Time from Inoculation.	Yeast Found, in Grammes.	Grammes of Sugar Fermented.	Ratio of Yeast to Sugar Fermented.	Yeast Found, in Grammes.	Grammes of Sugar Fermented.	Ratio of Yeast to Sugar Fermented.
42 hours	0.0985	0.531	1 : 5.4	0.1040	2.334	1 : 22.4
66 hours	0.1785	4.026	1 : 22.5	0.1692	4.364	1 : 25.8
90 hours	0.1900	6.812	1 : 35.8	0.2090	6.920	1 : 33.1

These distinctly show that when yeast is added to a solution of cane sugar, the yeast is employed for a considerable length of time in inverting the sugar before it commences to exercise its fermentative functions; but when a considerable quantity of sugar has become inverted, fermentation proceeds with much the same rapidity as it does in a solution of glucose.

Brown concluded that as Pasteur had not correctly determined the fermentative powers of the yeasts, and that as these are not comparable with each other, his theory remains without proof: "If, in the place of M. Pasteur's theory that fermentation is 'life without air,' is substituted the consideration that yeast-cells can use oxygen in the manner of ordinary aërobic fungi, and probably do require it for the full completion of their life history, but that the exhibition of their fermentative functions is independent of their environment with regard to free oxygen, it will be found that there is nothing in the results of any of M. Pasteur's experiments contradictory to such a hypothesis."

Experiments of H. Buchner and R. Rapp.¹—These authors point out that Pasteur's theory of fermentation is correct in a biological sense, the yeast organism having acquired the power of fermentation as an expedient function to replace respiration under certain conditions. They find, however, that the presence of free oxygen exerts no more influence on fermentative activity as such, *i.e.*, on the production of zymase, than does that of hydrogen or nitrogen; free oxygen simply favours cell multiplication. On the other hand, the fermentative property acquired by yeast by adaptation has become a permanent function, which it still exercises even in a purely aërobic existence in which fermentation would appear to be useless and superfluous. It is only when yeast is cultivated on solid (sugar-gelatin) media that it causes a strong respiratory decomposition of sugar, together then, however, with a largely predominating fermentative activity. Pasteur's biological theory therefore requires modification, for it is not, as he thought, the failure of oxygen which brings about the phenomenon of fermentation, since even in presence of an excess of oxygen the fermentative functions far outweigh the respiratory functions.

Further Experiments of Adrian Brown.—Brown has recently described a fresh series of experiments² on yeast reproduction under various conditions with the view of ascertaining the cause or causes which check yeast development in neutral media. Cell reproduction is found to proceed at a maximum

¹ *Zeit. f. Biologie*, 1898, **37**, 82. ² *Journ. Chem. Soc. Trans.*, 1905, **87**, 1395.

rate when the number of cells present is a minimum, and the rate falls with a decreasing velocity as the number of cells increases. This appears to indicate that the arresting influence is not conditioned by the accumulation of injurious products of cell metabolism but that it is due to some influence which favours reproduction *ab initio*. Experiments on yeast reproduction in malt wort containing different quantities of alcohol showed that the effect of added alcohol to the extent of 3 per cent. on reproduction is very slight, and even with an alcohol content of 8.4 per cent, the growth of yeast is not entirely prevented; the alcohol content, of course, increases during the fermentation, the above numbers representing the alcohol present at the beginning of the experiment. It is also shown that the other non-volatile products of cell metabolism have no marked influence on yeast reproduction. In this connection the action of carbon dioxide was determined. Equal numbers of yeast cells were incubated in equal quantities of malt wort saturated with carbon dioxide and hydrogen respectively at atmospheric pressure. Samples of malt wort containing the same number of yeast cells were incubated under ordinary conditions of air exposure. The results showed that the rates of reproduction and the total numbers of cells formed were practically the same in the presence of carbon dioxide and hydrogen, but that under these conditions they were much less than the values obtained in the air experiment. From the fact that the presence of carbon dioxide produces the same effect on cell reproduction as that of hydrogen, Brown concludes that the former possesses little or no power of arresting cell reproduction. The result of the fermentation, carried out in presence of air, shows, however, that oxygen exerts a stimulating effect on reproduction. In a further series of experiments Brown investigated the effect of a current of air on yeast growing in malt wort. Two flasks of wort were used, the one being seeded with four times as many cells as the other. The rates of reproduction differed much less in this case than in a similar experiment carried out under ordinary conditions (*i.e.*, without a current of air). It is also shown that in presence of a continuous supply of oxygen, yeast multiplication appears to be determined by food-supply, and the conclusion is arrived at that yeast-cell multiplication under anaërobic conditions is conditioned and governed by oxygen originally at the disposal of the cell prior to the commencement of reproduction. This explains the rapid fall of reproduction and the arrest of the same owing to the exhaustion of oxygen; it also explains the observation that in a given volume of liquid the final number of cells is the same, irrespective of the number originally added since the available oxygen at the beginning of the experiment is constant; it also tends to explain the observation that if more cells are added to a volume of nutritive solution than the maximum number to which a normal growth would develop, reproduction does not take place, since each cell would have at its disposal a very limited amount of oxygen.

Investigations of Dr. Emil C. Hansen.—We now come to the epoch-making work of the Danish physiologist, Dr. E. C. Hansen, on the organisms of fermentation, which has placed our knowledge of these on an entirely

new and much more definite basis. His researches have led to the discovery of properties and peculiarities possessed by these organisms hitherto unheard of even undreamed of, the importance of which to the brewer it is hardly possible to estimate.

The happy idea occurred to this philosopher that, in order to investigate with any degree of exactitude the life conditions or the specific action of any one set of these organisms, it was first of all necessary to secure a supply of them in a state of absolute purity. Fully recognising the fact that the only way to effect this must be to start such a culture from a single organism, he set about to devise a method by means of which this could be accomplished.

The Dilution Method.—In his earlier experiments pure cultures from a single yeast-cell were obtained by a method similar to that which had been employed by Lister in 1878 for securing pure cultivations of lactic acid bacteria, and which is known as “the dilution method.” A number of organisms are distributed in a quantity of a culture liquid, and the number present in a measured drop ascertained by means of the hæmacytometer. Another drop of the same mixture is then added to a quantity of sterilised water sufficient to ensure that each drop of this diluted mixture shall contain, on an average, less than one organism. If then a number of flasks containing sterilised culture liquid are taken, and a drop of this mixture added to each of them, and if, after a sufficient time has been allowed to elapse for the organisms to develop, some of the flasks remain sterile, it is extremely probable that the remainder will have only received one organism each.

Instead of employing the hæmacytometer for counting the yeast-cells Hansen made use of a microscope cover-glass, the central portion of which was ruled in a series of squares. A small drop of the liquid containing the yeast was placed on this ruled portion, the glass turned over and placed on a moist chamber, and the number of yeast-cells in the drop counted under the microscope. If such a drop, containing twenty cells, were mixed with 40 c.c. of sterilised water, 2 c.c. of this mixture would contain, on the average, one cell. If, then, to the sterilised contents of forty flasks 1 c.c. of this mixture was added to each, presumably one-half of the flasks would, in course of time, show signs of infection, and the other half would remain sterile. It was found in practice, however, that this did not always happen, for sometimes, in spite of all precautions, two organisms would gain entrance to one flask. The difficulty was obviated by the discovery that, if the flasks were vigorously shaken, in those to which two yeast-cells had gained access the cells were almost invariably widely separated by the shaking, and the colonies which they subsequently formed made their appearance in two different parts of the flask. In this way an almost absolute degree of certainty was introduced into the dilution method. It was by this modification that Hansen’s earlier observations were made, and upon these his principles were grounded.

Plate Cultivation.—In the year 1872 a method for securing pure cultures of bacteria was proposed by Schröter in which a semi-solid substratum was employed, and this, as improved by Koch, now forms the process chiefly used

for the isolation of bacteria. The method does not, however, provide that an absolutely pure culture is obtained with certainty; for, as shown by Miquel, 100 colonies obtained on a gelatin meat-broth plate may contain 108 different varieties; and Holm, working with yeast cultures on gelatin-wort plates, found that 100 colonies contained, on the average, 108 varieties and in extreme cases as many as 135.

Hansen's Modification of Koch's Plate Method.—Hansen, however, so modified the plate-cultivation method that it yielded absolutely certain

FIG. 57.—Bottcher Moist Chamber (*Hansen*).

results; it was so arranged that the individual yeast-cell was kept under observation during the whole time occupied in forming a colony.

Preparation of the Pure Culture from a Single Cell.—In Hansen's first method a dilute mixture of yeast and sterilised water is made in a sterilised flask. A drop of this mixture is examined under the microscope, and if the field contains more than twenty or thirty cells, more water is added until the proper degree of dilution is obtained. A test-tube containing sterilised wort-gelatin (prepared with clear bright wort of about 1056 specific gravity, and containing 5 or 6 per cent. of gelatin) is warmed until its contents are just fluid. A small portion of the yeast mixture is taken up on the platinum wire or loop and stirred into the gelatin, and, after the tube has been closed with a cotton-wool plug, it is turned and twisted about until the yeast-cells are evenly distributed throughout the gelatin. A drop of the mixture is then examined under the microscope to see if the dilution has been carried to the right extent—that is, that the individual yeast-cells are so far apart that there is no chance of the colonies they subsequently form touching one another. If this has not been secured more wort-gelatin is added. The right degree of dilution having been attained, a small drop of the gelatin is spread out in a thin layer on a microscope cover-glass and placed under a bell-glass until the gelatin has set; this takes place in about a quarter of an hour. It is then placed, gelatin side downwards, on one of the Bottcher moist-chambers shown in Fig. 57.

The chamber consists of a piece of glass tube about $\frac{3}{4}$ -inch in diameter and about $\frac{1}{2}$ -inch in length, ground perfectly true at both ends. One of these is cemented to an ordinary microscope slip; the other end is slightly smeared with vaseline, so that when the cover-glass is placed in position an air-tight joint is formed. A drop of water is placed in the chamber previous to its being used, in order to prevent evaporation from the surface of the gelatin. The whole is then placed under the microscope, and the miniature cultivation plate examined with a power of about 250 diameters. Those cells which are so far apart from one another that there is no chance of the colonies they

will subsequently form touching, are marked. This may be done by making small circles round the cells with an ordinary pen and ink, or with a small camel-hair pencil and a little Chinese white; or the object-marker of Klonne and Müller may be used. This last is an arrangement which screws on to the nose of the microscope in the place of the objective, and which, when the body of the instrument is pushed down, prints a little circle on the cover-glass. A cover-glass which has been previously marked out into small squares by means of hydrofluoric acid may also be employed, the columns of squares being numbered on two adjacent sides of the marked-out portion. It is well to make a sketch of the field, as this will materially assist in the subsequent identification of the cells. After the individual yeast-cells have been marked in one of these ways, and a number appended by the side of each circle to indicate it, or the two columns which intersect to form the square noted, the moist chamber and its contents may either be left at the ordinary room temperature or placed in an incubator and kept at a temperature of 24° to 25° C. (75° to 77° F.); in the former case the development of the colonies will be somewhat slow. The growth of the yeast-cells is watched day by day to see that the colonies do not encroach upon one another. In a few days they will have become so large that they can be seen with the naked eye. When they have arrived at this stage, portions of them are removed from the gelatin film and placed in wort in the following manner:—A piece of thin platinum or iron wire about half an inch long is taken up with a pair of tweezers and heated to redness in a Bunsen burner, and, after being allowed to grow cold, its end is inserted into one of the colonies and gently stirred about, so that some of the yeast-cells adhere to it. The wire is then dropped into a Pasteur flask containing sterilised wort. The flask, after being labelled, is placed in an incubation-chamber and kept at a temperature of from 25° to 28° C. (77° to 80° F.). In two or three days the contents of the flask will be found to be in a state of lively fermentation, and in a week or so a fair quantity of yeast will have formed.

Some of the Diseases of Beer may be Caused by the Saccharomycetes.—When pure cultures of yeast, obtained in this way from a single cell, came to be investigated, it was found that the ordinary yeast used in breweries never consisted of one single species, but of a number of varieties exhibiting markedly different properties. This led to the important discovery that certain of those deteriorations which beer is liable to undergo in the course of its manufacture, and which up to that time had been solely attributed to the presence of those bacterial organisms commonly known as “disease ferments,” could be produced by some varieties of the yeast itself.

Distinction of the Different Varieties of Yeast.—Hitherto yeasts had been solely differentiated by the various forms they presented when observed under the microscope, but it was soon discovered, by working with pure cultures of one single species, that this criterion could not be relied on. Although a circular or slightly oval shape is to a certain extent characteristic of the *Saccharomyces cerevisiae*, an oval one of the *S. ellipsoideus*, and a

sausage-shape of *S. Pastorianus*, yet under certain circumstances any one of these varieties may temporarily assume the forms of the others. Hence it became necessary to discover some test or tests by which the different varieties of yeast could be distinguished with certainty. This problem was also solved by Hansen, who, by making observations on the occurrence of spore formation at different temperatures, found that each species only developed spores between certain definite temperatures, and that when the whole of the six races were cultivated at a definite temperature at which they were all able to form spores, much difference was exhibited in the time which elapsed before spore formation commenced in the various species. By taking the times of the development of spores as abscissæ, and the temperature as ordinates, it was possible to construct a curve for each yeast, and these curves differed materially from one another.

A number of organisms which had hitherto been included amongst the *Saccharomycetes* were found not to yield spores, such as *S. apiculatus*, *S. exiguus*, *S. mycoderma*. This pointed to a great difference in the nature of these organisms and that of the true *Saccharomycetes*. In testing yeasts from various sources Hansen found that there were three distinct species of spore-bearing *Saccharomycetes*, and that each of these species could be further subdivided into varieties. The variety was indicated by placing a Roman numeral after the name of the species: thus we have *S. cerevisiæ I.*, *S. cerevisiæ II.*, and so on. Their general forms are illustrated in the accompanying figures (58 to 63), and their temperature limits in the tables appended.

FIG. 58.—*Saccharomyces cerevisiæ I.* (Hansen).

At 37.5° C.	no ascospores are developed.		
„ 36–37° C.	the first indications are seen after 29 hours.		
„ 35° C.	„ „ „	25	„
„ 33.5° C.	„ „ „	23	„
„ 30° C.	„ „ „	20	„
„ 25° C.	„ „ „	23	„
„ 23° C.	„ „ „	27	„
„ 17.5° C.	„ „ „	50	„
„ 16.5° C.	„ „ „	65	„
„ 11–12° C.	„ „ „	10 days.	
„ 9° C.	no ascospores are developed.		

Size of the spores, 2.5–6 μ .

The temperature at which the divergences are widest with these six varieties is about 52° F., at which ten days are required by *S. cerevisiae* to form spores, less than four by *S. Pastorianus I.* and *II.*, less than seven by *S. Pastorianus III.*, less than four and a half by *S. ellipsoideus I.*, and less than four and a half by *S. ellipsoideus II.* In order to find out if a given sample of yeast is contaminated with any of the last five varieties—or, as they are

FIG. 59.—*Saccharomyces Pastorianus I.* (Hansen).

At 31.5° C. no ascospores are developed.				
„ 29.5°–30.5° C.	the first indications are seen after 30 hours.			
„ 29° C.	„ „ „	27	„	
„ 27.5° C.	„ „ „	24	„	
„ 23.5° C.	„ „ „	26	„	
„ 18° C.	„ „ „	35	„	
„ 15° C.	„ „ „	50	„	
„ 10° C.	„ „ „	89	„	
„ 8.5° C.	„ „ „	5	days.	
„ 7° C.	„ „ „	7	„	
„ 3°–4° C.	„ „ „	14	„	
„ 0 5° C. no ascospores are developed.				

Size of the spores, 1.5–5 μ .

termed, wild yeasts—it is only necessary to cultivate a portion of it on a plaster block at a temperature of 52° F., and, if no spores make their appearance before ten days, the sample may be pronounced free from wild yeasts. In making such a culture it is necessary that Hansen's directions be rigidly adhered to, or the spore formation will be irregular; he has shown that if the young cells of a particular sample of yeast are cultivated in the same wort for two days instead of one before being transferred to the plaster block, the time of spore formation will not be the same.

Of the varieties of yeast capable of producing diseases in beer, *S. Pastorianus I.* communicates an intensely nauseous bitter flavour to beer, and *S. Pastorianus III.* and *S. ellipsoideus II.* cause persistent cloudiness. *S. Pastorianus II.* produces no ill effects in beer, neither does *S. ellipsoideus I.*, which is the principal yeast occurring in the fermentation of grape-juice.

Introduction of Pure Cultures of Yeast into the Brewery.—In 1883 Dr. Hansen published a paper in which he described the removal of an irregularity which had for two years shown itself in the products of the Tuborg Brewery, near Copenhagen ; the beers of which were affected with persistent turbidity. This was effected by introducing into the brewery yeast grown from a single cell. At about the same time the beers of the old Carlsberg Brewery became affected with a nauseous bitter taste and unpleasant odour. The yeast in use there was examined and found to consist of four different varieties. After experimenting with these singly and in combination

FIG. 60.—*Saccharomyces Pastorianus* II. (Hansen).

At 29° C. no ascospores are developed.			
„ 27°-28° C.		the first indications are seen after 34 hours.	
„ 25° C.	„	„	25 „
„ 23° C.	„	„	27 „
„ 17° C.	„	„	36 „
„ 15° C.	„	„	48 „
„ 11.5° C.	„	„	77 „
„ 7° C.	„	„	7 days.
„ 3°-4° C.	„	„	17 „
„ 0.5° C. no ascospores are developed.			

Size of the spores, 2-5 μ .

it was found that only one of the four was capable of yielding a satisfactory beer. Another of them was found to be *S. Pastorianus* I., and this it was which had given to the beer its objectionable flavour and odour. The state of matters was rectified by the introduction of a pure culture of that yeast which gave the normal beer, and this same variety, which is now known all over the world as Carlsberg bottom yeast No. 1, has been employed there ever since. This introduction into the fermentation industries of yeasts consisting entirely of one race or species, obtained by starting the culture from a single cell, constitutes Hansen's great reform ; the benefits to be derived therefrom are obvious. Previously, brewers were completely in the dark as to the nature or properties of the organisms they were cultivating in their worts.

Now, it is comparatively easy to find the particular race or species of yeast best fitted for producing each class of beer, and having once obtained it, it is possible to secure ever afterwards a constant supply of yeast possessing the same identical characters and properties. (Compare, however, remarks on single-cell yeast and top-fermentation beers, p. 386.)

Difference in the Properties of Various Yeasts.—When the method of making pure cultures of yeast from a single cell began to be extensively practised, it was soon discovered that yeasts differed remarkably in their properties. Thus some were found to attenuate wort much more rapidly than

FIG. 61.—*Saccharomyces Pastorianus* III. (Hansen).

At 29° C. no ascospores are developed.			
„ 27°–28° C.	the first indications are seen	after 35 hours.	
„ 26.5° C.	„	„	30 „
„ 25° C.	„	„	28 „
„ 22° C.	„	„	29 „
„ 17° C.	„	„	44 „
„ 16° C.	„	„	53 „
„ 10.5° C.	„	„	7 days.
„ 8.5 C.	„	„	9 „
„ 4° C. no ascospores are developed.			

Size of the spores, 2–5 μ .

others, some to yield beers of a much more stable nature than others. The flavours of beers, produced from a wort similar in all respects, were found to vary considerably when different races or varieties of yeasts were employed. Considerable difference was found in the amounts of the dextrins fermented away; in other words, some yeasts attenuated to a much higher degree than others. Such is the effect of the yeast upon the flavour of the fermented product that it has been found possible to obtain from malt wort, by employing for its fermentation one of the wine yeasts, a beverage scarcely distinguishable from wine.

FIG. 62.—*Saccharomyces ellipsoideus* I. (Hansen).

At 32.5° C. no ascospores are developed.

„ 30.5°–31.5° C. the first indications are seen after 36 hours.

„ 29.5° C. „ „ „ 23 „

„ 25° C. „ „ „ 21 „

„ 18° C. „ „ „ 33 „

„ 15° C. „ „ „ 45 „

„ 10.5° C. „ „ „ 4½ days.

„ 7.5° C. „ „ „ 11 „

„ 4° C. no ascospores are developed.

Size of the spores, 2–4 μ .

FIG. 63.—*Saccharomyces ellipsoideus* II. (Hansen).

At 35° C. no ascospores are developed.

„ 33°–34° C. the first indications are seen after 31 hours.

„ 33° C. „ „ „ 27 „

„ 31.5° C. „ „ „ 23 „

„ 29° C. „ „ „ 22 „

„ 25° C. „ „ „ 27 „

„ 18° C. „ „ „ 42 „

„ 11° C. „ „ „ 5½ days.

„ 8° C. „ „ „ 9 „

„ 4° C. no ascospores are developed.

Size of the spores, 2–5 μ .

The Practical Application of Pure Cultures of Yeast in the Brewery.—

In introducing a culture of single-cell yeast into a brewery, the sample of yeast being used at the time is taken and from it a number of single-cell cultures made. It is best for this purpose to take the yeast from the upper strata of the wort at the beginning of a fermentation, for it has been found that when the pitching yeast contains wild yeasts, the proportion of these as compared with the cultivated yeasts is then proportionally small. In those cases where the actual isolation of the yeast-cells has to be performed at some distance from the brewery, a sample of the ordinary yeast is forwarded. The operator begins by starting a fermentation with a little of the yeast in a Pasteur flask, and, after fermentation has fairly set in, a number of single-cell cultures are made with a little of the wort, which will now contain an abundance of young and vigorous yeast-cells. The contents of a number of large Pasteur flasks containing ordinary sterilised wort are then infected with the various colonies thus obtained, and after fermentation is completed in each, the resulting beer is examined as to taste, odour, clearness, &c. The yeast which has yielded the most promising beer is then developed until a sufficient quantity has been obtained to start an ordinary fermentation in the brewery. As this sample will have to be tried in actual practice before a definite opinion can be formed as to its suitability, the remainder of the flasks should be kept, so that, in case the sample taken into use does not prove satisfactory, the other yeasts may be tried in turn. With this object in view they should be placed in a dark cupboard, where they may be kept alive for a considerable time. The best preservative for yeast, however, is a 10 per cent. solution of cane sugar; in this, yeast will preserve its vitality for seven or eight years.

Small quantities of yeast may also be preserved in sterilised filter-paper. For this purpose a speck of yeast is placed on a piece of filter-paper which has been sterilised in a hot-air oven, and which is, immediately before being used, passed through the flame of a Bunsen burner. It is then wrapped up, and enveloped with a few more layers of sterilised filter-paper and placed in a drawer. According to Hansen, samples of yeast may be preserved in this way for five months, and often for much longer. This is an exceedingly convenient method for transmitting samples by post.

The Production of Single Cell Yeast in Quantities sufficient for the Brewery.—For this purpose five Pasteur flasks are required, each of the capacity of a quart. Their contents are pitched with the selected yeast, after which the flasks are allowed to stand for a week at the ordinary temperature. Four metal vessels, each holding about two gallons, of the form shown in Fig. 64,¹ known as “Carlsberg vessels,” are also necessary. These latter are made of tinned sheet copper, and are something like an ordinary oil-can in shape. A short tube is provided at *a* for emptying the

¹ This and the following figures are taken from Dr. Hansen's “Practical Studies in Fermentation,” London, 1896, to which the reader is referred for fuller information on the subject of this chapter.

vessel of its contents; it is fitted with a piece of india-rubber tubing and a glass stopper, similar to those used in connection with the side tube of a Pasteur flask, and is, in addition, provided with an ordinary spring burette-

FIG. 64.—Carlsberg Flask (*Hansen*).
a, outlet tube; *b*, inlet tube; *c*, bent tube; *d*, mouth of can; *e*, chamber filled with cotton-wool; *f*, air-filter.

clip. Another tube, which serves for the introduction of liquids into the vessel, is inserted at *b*; it is also provided with an india-rubber tube and a glass stopper. A short tube is fixed on the mouth of the can *d*, to which, by means of a piece of india-rubber tubing, the curved side tube *c* is attached. In a more recent modification, the tube *c* is attached to the neck by a screw union joint, so contrived that an absolutely air-tight joint is secured. To the other extremity of the bent tube *c* a small brass cylinder is attached which can be screwed on and off; this is filled with cotton-wool, well pressed together, or with asbestos fibre; it is provided with a loosely fitting cover. The bulb at *e* acts as a safeguard against infection, especially just after the sterilisation has been effected, when the condensing steam is liable to cause a sudden

inrush of air. It is necessary that the vessel and all its various parts should be absolutely air-tight, so that no air is able to enter except that which passes through the air-filter.

Sterilisation of the Carlsberg Vessels.—In order to effect this, one of the vessels, after being thoroughly cleansed, is about half filled with water. A flame is then applied to its under surface, the water brought to the boil, and kept in a state of vigorous ebullition for an hour, the air-filter *f* and the stopper of the tube *b* being meanwhile removed. After an hour's boiling the stopper, having been passed previously through a Bunsen burner, is replaced in the tube *b*. The boiling is kept up for another quarter of an hour, during which steam rushes through the bent tube *c*. In order to secure the sterilisation of the tube *a*, shortly before the flame is extinguished its stopper is removed and about 100 c.c. of hot water allowed to flow away; it is then closed with the glass stopper, which has previously been made fairly hot in a Bunsen flame, and the clip replaced. The source of heat is now removed and the air-filter *f* screwed on, the tube *a* opened, and the bulk of the water allowed to run off; the little which remains in the vessel is of no consequence. One and a half gallons of wort are now introduced through tube *b*, the air-filter is removed, heat applied, and the boiling conducted in exactly the same manner as with the water; the only extra precaution to be taken is that while the 100 c.c. of wort are being run off from tube *a*, and during the next quarter of an hour in which the wort is being boiled, the bent tube *c* is strongly heated with a Bunsen flame. The sources of heat are

then removed, and the air-filter, which has in the meantime been sterilised in the hot-air oven, is screwed tightly into its place. Before one of these vessels is taken into actual use it must be kept for several days, and a sample of its contents then drawn off from tube *a* and examined. During this operation the bent tube *c* is heated with a Bunsen flame.

It has since been found, that when yeast is grown in one of these vessels, in which the wort is not saturated with air, the yeast produced does not give normal clarification for some time when subsequently employed in the brewery. It is necessary therefore that the wort should be aërated if a normal yeast is to be obtained; and this aëration may be affected by allowing the vessel to stand for some time before being used. But the aëration is much more quickly attained by removing the stopper from tube *a*, connecting with it a piece of sterilised glass tube, and forcing in air which has been passed through an air-filter. A much more effective aëration is, however, secured if air is passed through the wort while it is still hot; consequently, the better plan is to commence the passing of air through the wort as soon as the flame is removed from the vessel, and continue the aëration until the contents of the vessel have fallen to 30° or 35° C. (87° to 95° F.). During all this time a flame is kept playing on the bent tube *c*, and this is not removed until the wort has acquired the above temperature. The air-filter is then screwed on. According to Nielsen's experiments, 60 litres (2.1 cubic feet) of air passed through the wort, at such a rate that the time occupied by its passage shall be about five or six hours, suffices to effectually aërate the contents of one of these vessels.

Of the five large Pasteur flasks, which should now contain a vigorous cultivation of the selected yeast, four are used for the subsequent pitching of the wort in four Carlsberg vessels, the fifth being kept as a reserve in case of accident. One of the flasks is then taken, the bulk of the liquid poured off from the deposited yeast, and the flask gently shaken so as to bring the yeast into suspension in the remainder of the fermented wort. This mixture of yeast and wort is then introduced into one of the Carlsberg vessels, all necessary precautions being taken to avoid contamination from the external atmosphere. The remaining three flasks and three Carlsberg vessels are treated in the same way. Fermentation will have thoroughly commenced in all of these by the next day, when the air-filter may be removed from each. The fermentation may be hastened by gently shaking the vessel occasionally, so as to remove a portion of the carbon dioxide gas, but, before doing this, the bent tube should be heated in a Bunsen flame. In the course of about seven days as much yeast will have been formed as can be produced under these conditions, and the four vessels, which will collectively contain sufficient yeast to pitch a hectolitre (22 gallons) of wort, may then be taken to the brewery. Here a small vat provided with a loosely fitting cover, and having a capacity of about 36 gallons, is thoroughly cleansed, and about 22 gallons of the ordinary hopped wort being used in the brewery are run into it. After the exterior surfaces of the Carlsberg flasks have been singed with a Bunsen

flame, they are gently shaken up so as to bring the yeast into suspension, and their contents poured one after the other into the wort. When the contents of the vat are in a lively state of fermentation and yeast begins to appear on the surface, the whole may be added to three or four hectolitres of wort (66 to 88 gallons) contained in a larger vat ; or a larger vat may be used at the commencement, 22 gallons of wort being run into it and pitched with the contents of the four vessels, and after its contents are in an active state of fermentation, another 70 or 80 gallons of wort are added. The yeast from this is collected, weighed, and used for pitching an ordinary brew. In working with the smaller quantities of wort it is advisable to carry on the fermentation at a temperature slightly higher than that usually employed in the brewery.

Pure Yeast Cultures occasionally Work Irregularly at first.—Hansen directs attention to the fact that the yeast produced in this way occasionally gives, at first, a higher degree of attenuation than the normal, and that it does not yield such a brilliant beer as it will subsequently. He cautions brewers not to be alarmed if these appearances show themselves, as they will disappear after the yeast has passed through two or three fermentations.

The Pure Cultivation Apparatus.—When pure yeast is introduced into a brewery it is exposed to a number of contaminating influences, in consequence of which it becomes sooner or later impure. The length of time which yeast will remain in a comparative state of purity differs much with the various species and races of yeast, but the most resistant succumb after a time. It is, therefore, far better to be provided with some means of keeping up a constant and regular supply of pure yeast, than to be obliged to go through the laborious process just described each time a change of yeast is required, and for this purpose various forms of apparatus have been contrived. The first of these, which was devised by Hansen and Kühle, and introduced to public notice in 1887, is now employed in hundreds of bottom-fermentation breweries, and has proved itself a thoroughly reliable apparatus ; all the other forms may be simply regarded as modifications of this. The original apparatus is shown in the accompanying illustration (Fig. 65). It consists of two principal portions, a closed fermenting vessel, *C*, and a closed wort-sterilising vessel, *D*. Besides these, there is a large metal cylinder, capable of withstanding a pressure of 100 lb. to the square inch, furnished with a pressure-gauge and a safety-valve, and also an air-pump, shown to the right in Fig. 66. By means of the latter, air drawn through an air-filter is forced into the cylinder until a pressure of three or four atmospheres is obtained ; this supply of compressed air serves, in the first instance, for the aëration of the wort while in the sterilising vessel, and afterwards for aërating it when in the fermenting vessel. The wort cylinder, *D* (Fig. 65), has a cover which can be screwed down air-tight, and into this two taps are inserted. One of these is furnished with a tube reaching about three-fourths of the way down the vessel, as shown by the dotted lines, to this tap the air-filter *m* is attached, and to the other the bent tube *n*, the extremity of which dips into the vessel *o*, which is

FIG. 65.—Hansen's Pure Yeast Culture Apparatus.

C, fermenting vessel ; *D*, wort sterilising vessel ; *b*, rouser ; *c*, bent tube ending in vessel *d*, which is partly filled with water ; *e*, upper tap of gauge ; *f*, gauge ; *g*, air-filter ; *h*, inlet tap for air ; *j*, tube for introduction of pitching yeast ; *k*, pipe and tap connecting steriliser and fermenter ; *l*, tap for drawing off yeast and wort ; *m*, air-filter ; *n*, bent tube connecting steriliser and vessel *d*, partly filled with water ; *o*, tap for indicating height of wort ; *r*, tap for drawing off wort ; *s*, tap connected with the ordinary wort-main.

nearly filled with water. This tube should not be less than half an inch in diameter, or it is liable to be blocked up by pieces of hop. Four other taps are inserted into the body of the vessel; the one communicating with the pipe *k* serves for conducting the sterilised wort to the fermenting vessel. The tap at *s* is connected with the ordinary wort main of the brewery which leads from the copper to the cooler. The tap *q* serves to indicate when sufficient wort is in the cylinder, and the tap *r*, inserted in the bottom of the cylinder, is used for drawing off the first portions of the wort introduced, which are diluted with the condensation water from the steam used in sterilising. The cylinder may be provided either with a circular pipe immediately under its lid, having a number of small holes on its inner side through

FIG. 66.—General View of Hansen's Apparatus.

which cold water is passed, and which, after streaming down the outside of the vessel and cooling its contents, is caught in a trough at its base; or the cylinder may, as shown in the figure, be supplied with a mantle, through which a supply of cold water is passed. The whole arrangement is fixed at a higher level than the fermenting vessel, so that the wort will flow from the one to the other by gravitation.

The fermenting cylinder *C* is similar in shape to the wort cylinder, and is also provided with a removable air-tight cover. To the centre of this lid a stuffing-box is attached, through which passes the axle of the rouser *b*, one end of which is provided with a handle, while the other is supported in a socket. To its lower extremity the blades of the rouser are attached, one of which is provided with a piece of sheet india-rubber, which sweeps the bottom of the vessel when the rouser is put in action. A tap is also inserted in the lid, on which the bent tube *c* screws. The lower extremity of this

tube is bent into a half circle, and is immersed in the vessel *d*, which is partially filled with water. To the right of the cylinder will be seen the gauge *f* which serves to indicate the height of the liquid in the vessel; it is marked at several points. The topmost mark indicates 31.6 inches from the floor of the cylinder, and the vessel, when filled up to this, contains about thirty-seven gallons. The mark below this is at a distance of eight inches, and the lowermost four inches above the floor of the vessel. The pipe *kk* leading from the wort cylinder enters at the bottom of the fermenting cylinder; in addition to the taps shown in the figure, there are two others connected with the pipe *kk* (shown in Fig. 66), one for admitting high-pressure steam, the other for allowing the escape of oil or steam. In the middle of the fermenting cylinder at *j* there projects a small tube about three quarters of an inch long, provided with an india-rubber tube and glass stopper; it serves for the introduction of the pitching yeast. The tap *l*, seen to the left, serves for drawing off the yeast and wort, its screw valve is raised or lowered by the handle underneath, and the tap is so contrived as to render contamination from the outside atmosphere impossible when the vessel is being emptied. Communicating with this tap there is a pipe which projects into the interior of the vessel and bends downwards, terminating at a distance of one and a half inches from the bottom of the cylinder. This arrangement prevents the entrance of air into the cylinder through the tap *l* when it is being emptied. The exterior of the vessel may be clothed with wood, or, as shown in the figure, surrounded with a mantle through which cold water for attemperating may be passed.

Air-Filters.—These, which are represented in the engraving at *m* and *g*, consist of metal tubes, one and a quarter inches in diameter and nine inches long, and are closely packed with cotton-wool, at least one and a quarter ounces being used for this purpose. They are provided with metal lids which screw on, each lid carrying a union joint, by means of which it may be placed in communication with the pipe from the air-vessel. The air-filters, before being used, are sterilised in the hot-air oven, where they are allowed to remain for two hours at a temperature of 150° C. (302° F.).

Method of Working Hansen's Apparatus.—Before being used, the apparatus must be carefully tested to ascertain that it is perfectly air-tight; this may be done either with steam or water under pressure. The next thing is to sterilise the two cylinders and all the communicating pipes, taps, &c., with steam. The fermenting cylinder (Fig. 65) is first sterilised by admitting steam to the pipe *kk*, and opening the tap which admits steam to the cylinder *C*, the air-filter *g* having been previously removed. The various other taps, *e*, *h*, *l*, *c*, are opened in turn, one after the other, so that they and the bent tube *cc* are all effectually sterilised; this takes about half an hour. The taps *e* and *h* are then closed, the filter *g* screwed on to its place, and all the other taps, with the exception of *c*, shut off. The tap from the air-vessel to the filter *g* is now opened and also the tap *h*, so that a current of sterilised air may flow into the cylinder through *h*. The steam is then very gradually shut

off, care being taken that steam mixed with air escapes from the end of the bent tube *c* for some time. As the admission of steam becomes less and less the cylinder gradually cools, and when steam has nearly ceased escaping from *c*, the vessel *d* is partially filled with water and the tap *k* closed. If the apparatus is cooled too suddenly, air will be drawn in through the bent tube by the vacuum formed by the condensation of the steam, or a pressure may be developed sufficient to cause the vessel to collapse. The cooling should occupy about two hours. The water in the vessels *d* and *o*, in which the bent tubes are inserted, serves to show that a slight air pressure is being maintained in the vessels. The wort cylinder is sterilised in a similar manner, but here there is no occasion for the slow cooling; as soon as the vessel is sterilised the tap *s* is opened, and hot wort allowed to flow into the cylinder until it begins to run out at the tap *q*. The air and steam above the wort escape partly by *q* and partly by the bent tube *n*. It is advisable to remove the first portions of the wort which enter the cylinder by the tap *r*, as these are diluted by the water that has condensed during the steaming-out process. When the cylinder is filled to the proper height the taps *q* and *s* are closed, and air allowed to bubble through the wort by means of the pipe communicating with the air-filter *m* for an hour (hot aëration). The wort is then cooled by passing cold water through the mantle, and when its temperature has been reduced to 60° F. it is admitted to the fermenting cylinder *C* through the pipe *k*. The fermenting cylinder is, at first, only filled so far that the level of the wort is a little below the aperture *j*, through which the pure culture of yeast from a large Pasteur flask is then introduced, all the usual precautions against infection from the outside being taken, the flame of a spirit-lamp being used for singeing, if a Bunsen flame is not at hand. The rouser is then set in motion and the yeast well mixed in, after which wort is run in from vessel *D*, until its level reaches the highest mark on the gauge. During the fermentation it is advisable that the wort should be aërated; the supply of air being so regulated that a small bubble escapes every few minutes from the end of the tube *c*. In a week or ten days, according to the temperature at which the fermentation has been conducted, the newly-formed yeast may be withdrawn from the apparatus. In order to do this the tap *l* is opened and the beer allowed to run off until foam begins to appear. The tap is then closed, and sterilised wort from the wort cylinder run in to the lowest mark but one on the gauge. The rouser is set in action, and the mixed wort and yeast drawn off into a clean vessel until the level of the liquid has sunk to the lowest mark on the gauge. This operation is repeated, and in this way eighty-eight gallons of wort, containing sufficient yeast to pitch five barrels of wort, are obtained. Enough yeast is left in the apparatus to start the next fermentation after it has been again filled with wort. Care must be taken during all the time the drawing-off of the contents of the vessel proceeds that air passes in through the air-filter *g*, in quantity sufficient to prevent any chance of the liquid in the trough *d*, or of air, being drawn through the bent tube *c*. Sterilised wort is again admitted into the cylinder until the

topmost mark on the gauge is reached ; the whole is then well roused, and a fresh fermentation started.

Apparatus of Bergh and Jörgensen.—This is a very efficient arrangement and occupies less space than the apparatus just described. It is so constructed that the wort is sterilised in the same cylinder that the fermentation afterwards takes place in. The apparatus, which is shown in Fig. 67, consists of two cylinders, **A** and **B**, one placed above the other, and an air-vessel (not shown) communicating with the tap *Y*, situated immediately above the air-filter *D*. A pipe is continued down the side of the apparatus, and in its descent gives off three branches, each of which is commanded by a three-way tap, *A*, *B*, *C*. The topmost cylinder, **A**, holds about ten gallons; it is provided with a rouser, *E*, an air supply through the tap *A*, a tap *F* and bent tube, and a small opening, *a*, for the introduction of yeast and the withdrawal of samples. The upper and lower vessels are connected by the tube *P*, on which is fixed the tap *G*. The lower cylinder **B** has a closely fitting lid, through which passes the air-pipe from the tap *B*, and the axle of the rouser *I*, which is actuated by the bevel-wheels; the float *L* serves to show by the pointer outside the level of the liquid inside. The lid also carries the tap *K*, to which is attached a bent tube. A circular pipe, *M*, perforated with fine holes, surrounds the lower chamber immediately under its cover, and supplies the refrigerating water. The upper portion of **B** is enveloped in a mantle for cold water, the lower portion with another mantle for high-pressure steam, by means of which the contents of the vessel are boiled and sterilised. In the floor of the lower cylinder is the tap *Q*, communicating by means of the pipe *b* with the wort main of the brewery from which the vessel is filled.

FIG. 67.—Propagating Apparatus of Bergh and Jörgensen.

When the apparatus is about to be used, the lower chamber **B** is filled to the proper height with wort, steam is admitted to the under-mantle, and the wort vigorously boiled, the taps *B* and *A* being placed in such a position that the steam passing away from the wort sterilises the upper chamber as well. The wort is then cooled and aërated, the yeast added through *a*, and a little

wort forced into the upper chamber by means of the compressed air, roused well with the added yeast, and allowed to run down into the lower cylinder. When the yeast is all in the lower chamber the tap *G* is shut and fermentation allowed to proceed. A good crop of yeast having formed, the contents of vessel **B** are well roused, and a portion of the fermented wort with the yeast in suspension is forced up into the vessel **A**; this serves for starting the next fermentation. The remainder of the wort and yeast are then run off and used for pitching in the brewery. In this way the vessel **B** serves in turn for a wort steriliser and for a fermenting vessel.

Advantages of Hansen's System.—The principal advantage claimed for this system is the introduction of an element of certainty into the process of fermentation which previously was never attainable. Before the introduction of this great reform, it has been urged that the brewer was in complete ignorance as to the nature of the yeast with which he was conducting his fermentations. In all cases, excepting those where a pure cultivated yeast is employed, the pitching yeast consists of an unknown mixture of various types and races, which, according to the laws of competition, are continually changing with every variation of the surrounding conditions in which the yeast is cultivated. At times the yeast becomes so degenerated that it will no longer fulfil its functions properly, and a change of yeast has then to be obtained from some other brewery, where probably little or no better conditions reign. Consequently, though such a change is often attended with benefit, yet in some cases the new yeast gives worse results than the old.

Under the reformed system it has been pointed out that the brewer can make choice of the one individual race or species of yeast which he finds best adapted to his requirements, and having once secured this, he is afterwards able to provide himself with a continuous supply of absolutely the same yeast. Among other advantages which have been claimed for the reformed system are constancy of flavour of the beer, ready clarification, and stability. It is also said to afford absolute protection against the diseases of beer and their attendant pecuniary losses; and to obviate the necessity of obtaining periodical changes of yeast from other breweries, with its attendant uncertainty. The Continental brewers, who employ low- or bottom-fermentation yeast, were the first to avail themselves of Hansen's method, and, as a consequence, the system is now, and has been for a good many years, employed with the most satisfactory results in hundreds of Continental and American breweries.

Hansen's System and Top-Fermentation Brewing.—As regards the application of single-cell yeast to breweries working on the high- or top-fermentation system opinions are somewhat divided. W. R. Wilson, C. F. Hyde, A. K. Miller, Julian L. Baker, and A. J. Murphy are among those who have expressed a favourable view of the results obtained when the system is applied to English beers. On the other hand Horace T. Brown and the late G. H. Morris, who were probably the first to try the system on an extensive scale in a large Burton brewery, found themselves compelled to give it up because they were unable to obtain a satisfactory secondary fer-

mentation, so necessary for bringing stock ales into condition. This, of course does not apply to the same extent in the case of quick draught running ales. Still, without in any way condemning the system, it may be pointed out that all the top-fermentation breweries in England which at one time adopted the system have now given it up. Writing in the *Transactions of the Jenner Institute* in 1889 the late G. Harris Morris put the case as follows:—"When Hansen first attempted to put his ideas into practice, the objection was raised in Denmark and elsewhere that the use of a culture derived from a single cell would interfere with what is known as the after, or secondary fermentation of beer; but it would appear that this objection was ill-founded in connection with the systems of brewing adopted on the Continent. The same doubts were expressed with regard to its application to English brewing, and although it is claimed that, as on the Continent, the question has been satisfactorily answered, yet, in the writer's opinion and experience, this is not the case, and we still await the solution of the question of the successful application of pure yeast to the conditions of English brewing. It has been suggested that the solution lies in the direction of the employment of two or more selected races of yeast, one to carry on the primary fermentation, and the other the secondary fermentation; and many breweries have been working with more or less success on these lines. The fact, however, remains that, up to the present, no satisfactory system, capable of general application, has been devised, and the question still awaits solution. That the use of pure yeast in some form or other has a future before it no one can doubt; but the conditions of brewing which will allow of that employment being successful yet remain to be worked out."

In a paper read before the Institute of Brewing, Morris,¹ details the results obtained in practice by himself in conjunction with H. T. Brown, between the years 1885 and 1894, in a brewery at Burton-on-Trent. During this time as many as 2000 barrels were brewed with one variety of yeast alone, and fresh varieties were continually isolated from the brewery yeast and put to practical test, the result being that the beers were, as a rule, sound and good in flavour, but remained thin and flat, and no secondary fermentation occurred unless after the addition of cold water malt extract or of ordinary sugar priming. Dry hopping also had a certain effect after long storage, due in part to the diastatic power of the hops, and in part to the introduction of wild yeasts with hops. In certain cases, vigorous secondary fermentations, amounting sometimes to a fret, took place, but this could always be traced to infection from outside.

R. L. Siau² has published his experiences of the use of single-cell yeast in an English top-fermentation brewery, and these quite confirm those of Messrs. Brown and Morris. He states that the use of single-cell yeast in practice is to be avoided in the preparation of a beer that is to be kept more than a month after racking. For the quickest kind of running trade ale (brewed

¹ *Journ. Fed. Institute of Brewing*, 333, 6, 1900.

² *Journ. Inst. Brewing*, 118, 12, 1906.

racked, and drunk within a month), the system supplies all that can be desired, and affords a means of obtaining that consistency of type and character which is so seldom secured. He shows that there can occur in beer fermented with single-cell yeast the continued fermentation which persists for about a fortnight. It is evidently to such a fermentation that W. R. Wilson and A. K. Miller referred when they spoke of the occurrence of a secondary fermentation in beers fermented with single-cell yeast, whilst, on the other hand, Brown and Morris and the Burton School apparently understand by the term secondary fermentation, one which does not set in until a month or six weeks after racking. Confusion has, therefore, arisen due to the same expression being employed for two totally different things, the arguments being maintained from opposite premisses.

The fact that primary yeast is unable to effect the secondary fermentation of English ales has been confirmed by the researches of N. H. Claussen¹ who discovered a non-sporulating, budding fungus, belonging to the *Torula* group, which, he claims, possesses the special property of setting up a secondary fermentation in such a way as to create the flavour and condition typical of English beers. This organism to which he gives the name *Brettanomyces* exists in several varieties, some of which are harmful. It produces a slow fermentation in wort or in beer fermented by ordinary brewers' yeast; the carbon dioxide produced by its action is retained firmly and conduces to the formation of a copious and lasting foam. A somewhat considerable amount of acid is formed, accompanied by ethereal substances, which at once call to mind the flavour of stored beer. If a few drops of a pure culture of *Brettanomyces* be sown in a bottle of Pasteurised beer and stored at a temperature of 75–85° F. for ten to fifteen days, the beer acquires the English character. The beer employed for this purpose should have reached a certain limit of attenuation during primary fermentation. Claussen believes that *Brettanomyces* is a constituent of English pitching yeast, otherwise it may enter the beer at some stage of the brewing process as an infection, and that its presence is indispensable for the secondary fermentation. He has taken out patents for the employment of *Brettanomyces* in English brewing, and he proposes that the question of single cell yeast for English beers should be reopened, that the primary fermentation should be conducted with pure beer yeast, and that pure cultures of selected varieties of *Brettanomyces* should be employed for the secondary fermentation.

Analysis of Yeast by Hansen's Method.—It is highly necessary, where pure cultivated yeast is employed in a brewery, to have some means of determining when the yeast in actual use is becoming so contaminated that a new supply from the propagating apparatus should be introduced, and also of ascertaining that the apparatus itself is fulfilling its duties properly. This is effected by making a spore cultivation on a block of plaster of Paris at a definite temperature, and noting the time when spore-formation makes its appearance. This varies with every species of yeast, and, according to the

¹ *Journ. Inst. Brewing*, 308, 10, 1904.

experiments of Holm and Poulsen, the two best temperatures for conducting experiments of this nature with low fermentation yeasts are 15° and 25° C. (59° to 77° F.). They state that low fermentation yeasts may be divided into two classes, one of which at the latter temperature yields spores later than the wild yeasts. At this temperature the other class sporulates at about the same time that the wild yeasts do, but at the lower temperature it forms spores some time after the wild yeasts.

The Yeast which is to be Examined Biologically should be taken at the End of the Primary Fermentation.—The sample is removed from the fermenting-room in a sterilised flask, and, after being allowed to settle for a few hours, is spread on the plaster blocks. The cultures carried out at 25° C. are examined at the end of forty hours, those at 15° C. at the end of three days. The method is said by Hansen to be so sensitive that an admixture of one two-hundredth of a part of wild yeast can be readily detected in the Carlsberg bottom-yeast. This is much beyond the degree of delicacy actually required in practice, for Hansen has shown that, when working under normal conditions and storage, one part of *S. Pastorianus III.* or of *S. ellipsoideus II.*, both of which in large quantities produce beer turbidity, are, in these small proportions, incapable of causing any ill effects, and that *S. Pastorianus I.* is only able to impart its disagreeable flavour and odour when it exists in a larger proportion than 1 part in 22 of an otherwise pure yeast.

Analysis of the Yeast of the Propagating Apparatus.—The purity of the yeast contained in this apparatus should be tested at the end of every fermentation. For this purpose a small portion is drawn off into a Pasteur flask, and cultivated for a few days at a temperature of 70° to 80° F. in yeast-water, when bacterial contamination, if present, will show itself. After being allowed to settle, the beer is decanted from the yeast in the Pasteur flask and the yeast passed through several cultivations in a solution of cane sugar acidified with tartaric acid, then a few times in beer-wort, after which it is tested by spore-formation on plaster blocks. This treatment with tartaric acid is exceedingly favourable to the development of wild yeasts and to the suppression of the cultivated varieties; consequently the former, if present in the sample, are brought into prominence.

Differences in the Actions of the Various Species of Yeast.—In addition to the broader differences between the members of the several races and varieties of the *Saccharomycetes* which have been already mentioned, many minor diversities are observable in the action even of varieties of the same species.

Differences in Fermenting Power.—The remarkable differences in the fermenting power of the various yeasts has attracted considerable attention lately. It was prominently brought into notice by the difference in the behaviour of two varieties of yeast, one of which had been isolated from the yeast of a brewery at Saaz, and is now known as Saaz yeast; the other from the yeast used in the brewery of Herr Froberg at Grimma, and which now bears the name of Froberg yeast. In conducting experiments with the

same wort, it was found that the Froberg yeast invariably carried the attenuation to a considerably lower point than the Saaz yeast did; the former attenuated a wort of sp. gr. 1049 to 1018, the latter was only able to reduce it down to 1023. It has now become customary to distinguish yeasts which possess high attenuating powers as Froberg types, and those which have more feeble powers in this direction as Saaz types; between these extremes there are many yeasts which hold various intermediate positions.

Since then another yeast, one of the *Schizo-saccharomycetes*, *Saccharomyces pombe*, has been discovered, which possesses an even greater power of attenuation than the Froberg yeast. It was found that it could reduce the original gravity of a wort 84 per cent., while the Froberg could only reduce it by 70 per cent.

This divergence is not confined to these yeasts, but is exhibited in a greater or less degree by all which have been examined so far. A number of experiments which were conducted by P. Lindner¹ on different yeasts demonstrate this very clearly. In each of these, 150 c.c. of sterilised wort were seeded with a trace of a pure culture of a particular variety of yeast, and this, when it had entered into a state of active fermentation, was added to a larger quantity of sterilised wort, generally about 1200 c.c. In each series of experiments the same wort was employed; some of the flasks employed were plugged with cotton-wool, others were closed with a bulb tube containing sulphuric acid. Consequently, in the former a slow interchange between the air of the flask and that of the outside atmosphere took place; in the latter this was effectually prevented. High and low fermentation, and also wild yeasts, were experimented with. Of the high fermentation yeasts examined, the one which exhibited the highest attenuative power reduced the gravity of a wort of 1049° to 1018°; the weakest in this respect only reduced the same wort to a gravity of 1024°. The remainder exhibited various attenuative powers intermediate between these extremes. Similar differences in attenuative power were observable with the low fermentation and wild yeasts.

The Differences in the Attenuative Powers of the Various Saccharomycetes on Wort are dependent upon the Nature of the Enzymes which they are able to secrete.—Whilst the vast majority of the yeasts are able to ferment the two sugars glucose and fructose, which require no previous inversion, this is not by any means the case with the disaccharide sugars maltose, cane sugar, and milk sugar. Certain yeasts, such as the *S. apiculatus*, do not secrete the necessary hydrolysing enzyme to enable them to ferment any of the disaccharides. Others, such as the *S. albicans*, Rees, are able to hydrolyse and ferment maltose, but not cane sugar. The *S. kefir*, Beyerinck, can hydrolyse and ferment cane sugar and milk sugar, but not maltose. Some yeasts, such as the *S. Ludwigii*, are unable to transform any of the sugars into alcohol, but appear to produce oxalic acid instead. These distinctions are also found in the mould fungi, and their ability to ferment the various disaccharides, or

¹ *Mikroskopische Betriebskontrolle*, p. 112.

the reverse, depends upon the same causes ; thus *Monilia candida* has recently been shown to secrete invertase.

The Yeasts commonly used in Brewing appear to be endowed with the Power of Secreting a Number of Different Enzymes.—In addition to producing the well-known enzyme, invertase, it has been recently found that the cultivated yeasts secrete another enzyme, maltase, which converts maltose into glucose previous to its fermentation. Evidently others are produced which have the power of degrading a greater or less number of those intermediary malto-dextrins which occur in wort, and to this are to be attributed the differences which are observable in the attenuative powers of the various yeasts. The power of hydrolysing and fermenting milk sugar seems to be entirely wanting in these yeasts.

Differences in the Behaviour of Various Yeasts to the Trisaccharide Raffinose.—It was shown by Berthelot, in 1889, that the various yeasts exhibited marked differences in their behaviour to this complex sugar ; the ordinary yeast of the French breweries (low fermentation) fermented it completely, whilst bakers' yeast (top fermentation) only fermented a third part of it ; and Loiseau found that this was true with respect to all bottom and top fermentation yeasts. In consequence of doubts which had arisen as to the validity of these observations, A. Bau reinvestigated the subject in 1894, and obtained the following results. It was found that top fermentation yeasts of the Froberg type hydrolysed raffinose into melibiose and fructose, but were only able to ferment the latter sugar. They were also able to ferment completely two bodies occurring in wort, which he termed α - and β -isomaltose.¹ Top yeasts of the Saaz type behaved in a similar manner towards raffinose ; they could only ferment α -isomaltose, but not the β -variety. The Froberg low yeasts completely hydrolysed raffinose into glucose, galactose, and fructose, all of which sugars they are able to ferment ; and this is also the case with α - and β -isomaltose. Saaz low yeasts behaved similarly with raffinose, but could only ferment β -isomaltose. In this way he founded a classification of the various yeasts according to their action on these various sugars.

Saccharomyces Thermantitonum.—Grove Johnson and S. Hare, in the course of some recent bacteriological experiments, accidentally came across a new yeast, which they isolated, and, owing to its power of resistance to high temperatures, they gave it the above characteristic appellation. It proved to be a true yeast, forming spores at definite temperatures, and capable of setting up a vigorous alcoholic fermentation in a saccharine solution at a temperature of 110–130° F. It was also found to have the power of resisting even much higher temperatures. This power of remaining alive at high temperatures was in fact utilised by the discoverers as a means of preparing pure cultures of the yeast. Experiments showed that when a liquid containing the yeast—which the discoverers for the sake of brevity refer to as S.T.—is raised to 180° F. for a short time, most of, if not all, the other organisms are killed, whereas the yeast itself is only weakened and may

¹ The existence of these two bodies is somewhat problematical.

be resuscitated by keeping it for a little while at 110° F. P. Lindner states that the new yeast differs from Logos yeast in that it precipitates in a much more compact mass. He further states that at 43° C. (109° F.), the fermentation proceeds quickly and normally, and the yeast retains its ability to isolate itself in large lumps, thereby causing a spontaneous clarification of the wort. He remarks, moreover, that it has divergent qualities from the so-called caseous yeasts of Matthews and Lott, and that he regards it as a distinct species. S.T. is a bottom yeast, and, as a matter of fact, it never rises to the surface of the fermenting liquid, like ordinary brewery yeast, but separates into small clots, which first rise with the liberated carbon dioxide, and at once sink again. Johnson and Hare assert that even when the fermentation of beer wort is proceeding rapidly, it may be seen that the wort is quite bright, apart from the small clots of ferment, and as soon as the fermentation is finished, the S.T. settles down in a hard compact mass, leaving the supernatant liquid absolutely bright.

The ferment has been found to be well adapted for brewing beer under the conditions prevailing in tropical countries, and the discoverers have carried out practical experiments with it in English breweries. They claim that beer can be produced without coolers or refrigerators, as it is quite easy to reduce the temperature of the worts to the degree necessary for pitching with S.T. by the attemperators contained in the fermenting vessel, and also that is not necessary to skim or cleanse, as the yeast sinks in clots to the bottom of the fermenting vessel after fermentation ceases. As the ferment is removed solid, no yeast pressing is necessary, and there are therefore no pressings to be mixed off. Fermentation can be stopped at any desired gravity by lowering the temperature, when the ferment lies dormant at the bottom of the vessel, and in this way a full drinking beer, low as regards alcoholic strength, may, it is stated, be produced from a light gravity wort. There is also said to be a saving of time; beer can be bottled within three or four days of brewing; dry hopping is unnecessary; and raw cane sugar can be used as well as invert, since this yeast ferments the former quite as readily as the latter. By taking advantage of the fact that S.T. resists a temperature of 180° F., it is easy to obtain a culture free from wild yeasts, &c. Finally, it is claimed that by dispensing with coolers, refrigerators, yeast presses and the pumps, mains and apparatus in connection with them, there will be a considerable saving in capital as well as in wages, whilst the loss of beer will be much reduced, the saving being estimated at 1s. 6d. to 2s. per barrel.

The following is quoted from the *Brewers' Journal*: "It would seem that Messrs. Johnson and Hare have discovered a new species of yeast, and so far their researches, from a technical point of view, are interesting; for a ferment of such distinctive characteristics may possibly find some practical application in the fermentation industries. The difficulties of producing satisfactory beers in tropical or even in sub-tropical countries are well known, and foremost among these is the question of cooling and attemperating. If anything approaching a drinkable beer, therefore, can be produced in hot

climates by means of S.T., a valuable application of it will be assured. But, admitting that fermentation with this yeast can be stopped at any desired gravity and brilliant beer obtained, would not the product be lacking in character, condition and stability? The necessity of thoroughly purging the wort in the fermenting vessel is well recognised by all practical brewers; whilst, in order to obtain satisfactory ales, secondary fermentation in cask is absolutely essential, even in the case of running ales. Judging from the particulars before us, secondary fermentation appears altogether ignored in the S.T. process. We need not emphasise this point. Suffice it to recall the experiences of Drs. Brown and Morris, which have now received ample confirmation, that a satisfactory secondary fermentation cannot be secured when a pure yeast is employed, and, indeed, as is well known, it is this fact which has stood in the way of the adoption of Hansen's system for English brewing. If it were feasible to stop fermentation before the wort had attenuated to its limit, a full drinking beer might be obtained from a wort of comparatively low gravity, but such a beer would only be fit for immediate consumption, and would undoubtedly fret in cask. We have had an opportunity of tasting some beers fermented with S.T., and whilst they were, we admit, quite sound, they lacked condition. The suggested use of cane instead of invert sugar is possible, of course, with ordinary yeast, but is not advisable, as it invariably leads to yeast weakness. We doubt not that S.T. may be turned to practical account in the manufacture of spirit or even of beer in the tropics, but as regards its application for the latter purpose in this country we are by no means convinced, knowing how much the character of beer depends on the system of fermentation and on the yeast with which it is fermented. It is, in fact, the highly divergent properties of S.T., as compared with ordinary yeast, which constitute its novelty; but this feature, interesting as it is, increases our scepticism as to its use in the production of English beer."

Variations in the Quantity of Alcohol Produced.—In connection with the series of experiments performed by P. Lindner, which have already been mentioned on p. 390, portions of a 29 per cent. solution of maltose were pitched with various yeasts in order to find the amounts of alcohol they were capable of yielding. The top fermentation yeasts were found to produce from 9 to 15 per cent. of alcohol by volume, and the low fermentation yeasts slightly less quantities. Saaz yeast yielded a little over 8 per cent., and Froberg not quite 11 per cent.

Quantities of Acid Produced by Different Yeasts.—Five top fermentation yeasts respectively yielded amounts of acid which, reckoned as lactic acid, varied from 0.117 to 0.135 per cent. of the wort. The Pombe yeast produces a much larger quantity of acid than this, and consequently has considerable power in keeping bacterial organisms in check.

Amounts of Nitrogenous Matter Removed from Wort.—This, with the various top fermentation yeasts, was found to range from 9.4 oz. to 13.8 oz. per barrel in a wort having a gravity of 1048.6. Low fermentation yeasts did not act so vigorously; they only removed from 5.64 to 11.52 oz. per

barrel from the same wort. Saaz yeast removed 7.03 oz. and Froberg 10.66 oz.

Reproductive Powers.—Yeasts vary much in the crops of yeast which they yield. In the same series of experiments the amounts of the new yeast formed varied from 9.25 to 23 grammes, the fermentation being started with a mere trace. In all but two instances the larger yeast crop was associated with a higher attenuative power. In those cases where parallel experiments were made, the one set in flasks closed with cotton wool, the other in flasks sealed with a sulphuric acid tube, the yeast crop and the attenuation were always greatest in the former, and this demonstrates the influence of even a slight amount of aëration.

Energy of Fermentation.—Leaving out of consideration the different physiological condition of the yeast, the various races show marked differences in their fermentative activity. This can be estimated by observing the amount of gas evolved in a given time, or by the time taken for a given amount of gas to be evolved. The latter method was that employed in P. Lindner's series of experiments, the measurement being indicated by the number of days taken to evolve 36 grammes of carbon dioxide. The number of days required by a number of the yeasts to do this varied from six to fifteen, but some of them did not evolve the quantity of gas during the whole period of the fermentation.

Hayduck's Apparatus for Ascertaining the Fermentative Energy of Yeast.—This consists of a flask, Fig. 68, *a*, for containing the yeast and a solution

FIG. 68.—Hayduck's Apparatus for Ascertaining the Fermentative Energy of Yeast.

of cane sugar; it is placed in a water-bath provided with a perforated false bottom, fixed about half an inch above the real bottom, the contents of the bath being kept at a constant temperature of 30°C . (86°F .). The flask is connected by means of an india-rubber tube with an apparatus for measuring the carbon dioxide evolved. The flask *a* is furnished with an india-rubber cork having two perforations, in each of which short pieces of glass tube are inserted. To one of these the tube communicating with the measuring apparatus is connected; to the other a short piece of india-rubber tube, which can be closed by an ordinary burette-clip. The measuring apparatus consists of a U-shaped tube, one limb, which is considerably wider than the other, is constructed to hold 500 c.c.; it is graduated into 10 c.c. divisions.

The extremity of the narrower tube carries a funnel-like expansion *f*, and is provided at its lower end with a tap *e*. The upper extremity of the wider limb carries a narrow tube bent at a right angle, and also a tap *d*. When a determination is made, 10 grammes of the yeast under examination are

intimately mixed with 400 c.c. of a 10 per cent. solution of cane sugar and introduced into the flask *a*. This is then placed in the water-bath, which has been previously heated to, and is now constantly maintained at, a temperature of 86° F., and is allowed to remain uncorked for an hour. In the meantime the measuring apparatus is made ready. Five c.c. of paraffin oil are first poured into the funnel *f*, and afterwards water. This latter drives the oil before it, and this, when it enters the measuring chamber, floats on the surface of the water, its object being to form a protective layer on the surface of the water, and prevent the absorption of carbon dioxide. Sufficient water is then introduced to bring the surface of the paraffin layer to the zero mark on the scale. After the flask has stood for an hour in the water-bath the apparatus is connected up, the tube *c* being left open while this is being done. This is now closed with the clip, the tap *d* opened, and the time noted. As fermentation proceeds the disengaged gas gradually displaces the liquid in the wider limb, and the tap *e* is opened just sufficiently to allow the water to escape at the same rate as the gas enters. At the expiration of half an hour the taps *d* and *e* are closed, the clip *c* is removed, and, with the assistance of the pointer, the level of the liquid in the two limbs adjusted at the same height, either by pouring water into the funnel or drawing it off by the tap *e*, and the quantity of the collected gas read off.

The fermentative activity of a yeast may be expressed by the number of cubic centimetres of gas it evolved in half an hour under these conditions; but the better and more usual method is to express it in terms of the number of grammes of sugar fermented by 100 grammes of yeast in half an hour. This is done by multiplying the number of c.c. of carbon dioxide collected during the experiment by the factor 0.03841 (which is obtained by taking into account the ratio of gas evolved to the amount of sugar fermented, the ratio of the weight of carbon dioxide to its volume), and multiplying the result by ten.

P A R T I I I

CHAPTER VI

WATER

Chemical Composition.—Water was, until comparatively recent times, regarded as an element, its compound nature being discovered by Cavendish in 1781, who showed that when hydrogen gas was burned in air, a quantity of water was formed equal in weight to that of the two gaseous elements which disappeared. Shortly after this Lavoisier proved that water was a compound of oxygen and hydrogen; and in 1805 Humboldt and Gay-Lussac determined its composition by volume. Chemically pure water consists of two parts by weight (two atoms) of hydrogen and sixteen parts by weight (one atom) of oxygen, or two volumes of the former gas and one volume of the latter. Of all known liquids water possesses the most extensive solvent powers; an immense number of different substances, liquid, solid, and gaseous, are readily dissolved by it. In consequence of this property, water is never met with in nature in a state of purity, the nearest approximation to this is the distilled water of the chemist's laboratory. Even this is not absolutely pure, for it contains a variable amount of dissolved gases derived from the air; indeed, the removal of the last traces of nitrogen is so difficult that it is doubtful if absolutely pure water has ever yet been prepared.

Occurrence.—Water is met with in nature in great abundance, where it plays an important part in most terrestrial phenomena. It is found in all the three physical states it is capable of assuming; in the solid state it occurs permanently in the polar regions and on the tops of high mountains as ice and snow, and in this state it appears periodically in temperate regions. In the liquid state it is met with in the ocean, lakes, seas, and rivers, &c., which constitute more than three-fourths of the surface of the earth; also under the earth's surface, where it forms the source of the supply of springs, wells, &c. The atmosphere is always more or less charged with water in the form of vapour, the amount which it contains at any one time being governed by local and general meteorological conditions.

In the economy of nature water is always in a state of constant circulation; its tendency, from whatever source it may be derived, is to flow back to the ocean, which may be regarded as an immense reservoir provided by

nature for its storage. From this store it is evaporated by the heat of the sun's rays and diffused in the form of vapour through the atmosphere, from whence it is again condensed, and appears in the form of rain, snow, hail, dew, &c. When precipitated to the earth in the form of rain, snow, &c., one portion flows off the surface of the land, giving rise to the various brooks, streams, lakes, rivers; a second portion evaporates; whilst a third portion sinks into the earth. This latter portion descends through the more porous portions of the soil until it reaches some impervious stratum by which it is retained to form the subterranean lake known as the "ground water," from which the water of surface wells is derived. The level of the surface of the ground water¹ varies considerably; in some districts it almost touches the

FIG. 69.—Diagrammatic Section of the Strata forming the London Basin.²

surface of the soil, whilst in other places it is situated at a considerable distance below the surface; hence the difference in the depths of wells in various districts. In hilly countries, where the strata are inclined, the underground water, obeying the natural laws of gravitation, forms subterranean watercourses, and these, when they find outlets in districts at a lower level, appear as springs. The accompanying diagram (Fig. 69) will help to explain these various conditions. The stratum *A*, in the middle of the basin, is sand, gravel, or some layer pervious to water. The rain, snow, &c., falling on this portion would be partially evaporated, a part would drain off the surface and fall into the river, *R*, and the remaining portion would sink into the soil until it reached the stratum marked *b*, which is clay, a substance impervious to water; here it would be retained and form the ground water. A well, *W*, sunk into this bed would consequently derive its supply from this ground water. The stratum *c* below the clay is the chalk; at *e* is the green-sand, whilst at *d* there is again an impervious stratum. As a consequence of this arrangement of the strata, a certain amount of the water falling on the exposed portions of *c* would sink into and be retained in this stratum, since, as *b* is impervious, no water can escape upwards, and as *d* also is impervious, no water can escape downwards. A well, *W*₂, driven into this stratum, would derive its supply from this pent-up body of water, and would con-

¹ The surface of the ground water is very far from being horizontal; as a general rule, it conforms to the inequalities in height of the surface of the ground.

² As with all geological sections, the height is greatly exaggerated in comparison with the width.

sequently have the character of a chalk water. The water derived from a well, W_3 , driven through the impervious layer d , would have the characters of a water coming from the greensand. Should the level of the water contained in the beds c and d be much higher than the surface of A , the water from these two wells would of itself rise above the level of the mouths of the wells; should it be lower, the water would only rise a proportional distance in the well and have to be pumped the remainder. From this it will be seen that the water obtained from a well may be collected at a great distance from the well itself.

Rain Water.—This, since it is derived directly from the condensation of aqueous vapour, is, when collected in the open country at a distance from human dwellings, the nearest approach to pure water met with in nature. It contains, however, many impurities derived from the air, for, in traversing the atmosphere, water dissolves an amount of oxygen, nitrogen, and carbon dioxide gases equal to about 0.05 of its volume; small quantities of ammonia, nitric acid, and sodium chloride are similarly taken up. In towns, rain water takes up sulphuric and sulphurous acid, the presence of which in the atmosphere is due to the burning of coal; sometimes hydrogen sulphide is also absorbed.

Besides these, rain water takes up small traces of the solid impurities which exist in the atmosphere in the form of dust. These are partly of an inorganic nature, such as calcium and magnesium compounds, or, more rarely, those of potassium, iron, and manganese; partly of an organic, such as portions of decaying vegetable tissue, the spores of bacteria and mould fungi, occasionally microscopical plants of the lowest orders (*Protozoococcus pluvialis*, &c.)

Rain water collected from roofs is generally very impure, since it contains much organic matter derived from the surfaces on which it has been collected, these being, as a rule, far from clean. When such water is stored in cisterns or other closed vessels it is apt to become putrid.

Waters from Wells and Springs.—Of the water which is precipitated on the surface of the earth in the form of rain, that portion which sinks into the ground varies very much, and is regulated by the nature, configuration, &c., of the ground on which it falls. In magnesian limestone districts about 26 per cent. finds its way into the subsoil; in the new red sandstone (Triassic), about 25 per cent.; in the chalk, about 42 per cent.; in the loose tertiary sand, as much as 90 to 96 per cent. After penetrating the soil, water absorbs a large amount of carbon dioxide from the air contained in the soil (the "ground air"), which is especially rich in this gas, containing, as it does, some 250 times more carbon dioxide than the air of the atmosphere. In descending farther it dissolves as much of the substances with which it comes in contact as it is capable of taking up, and it is much aided in this direction by the carbon dioxide which it holds in solution; thus when water saturated with this gas meets with chalk (calcium carbonate), the latter is converted into acid calcium carbonate, which is a soluble salt. But the decompositions which take place between water charged with carbon dioxide

and the constituents of the soil are often of a much more profound nature than this; for instance, many silicates undergo partial decomposition, small quantities of soluble alkaline silicates, alkalis, &c., being liberated. Double decompositions also often take place between the constituents of the soil, as when calcium sulphate and magnesium carbonate mutually decompose one another to form calcium carbonate and magnesium sulphate.

General Constituents of Well and Spring Waters.—Of the inorganic matters in solution, the most commonly met with are the following:—Bases: sodium, potassium, calcium, magnesium, and iron; acids and halogens; sulphuric, nitric, nitrous, and phosphoric acids; silica, and carbon dioxide. Of the organic matters taken into solution, some entirely consist of carbon, hydrogen, and oxygen; others contain, in addition to these three elements, nitrogen, and often sulphur, but we possess no very definite knowledge as to the exact nature of these bodies. Substances are sometimes met with in suspension, such as clay, &c. The respective bases and acids of the inorganic constituents are estimated directly by chemical analysis, but the organic substances present in solution can only be estimated indirectly.

Statements of Results of Water Analyses.—In these statements it is now customary to tabulate the metals found in terms of their oxides: thus calcium is expressed as lime (CaO), and the acids in terms of their anhydrides, sulphuric acid being expressed as SO_3 .¹ A second statement then follows, in which the various bases and acids are given in the state in which they are supposed to be combined in the water. Unfortunately, in tabulating the amount of the various constituents of a water, chemists employ very different methods of statements. Some express their results in grains per gallon, and probably for trade purposes this form of statement is the best, as every one has some notion of the weight of a grain and the quantity of a gallon. This has been objected to on the ground that a decimal form of statement is a more scientific one, and this view of the subject has led to the results of water analyses being stated in parts per million, parts per 100,000, and in some few cases in parts per 1000. Of these different methods, the first, which corresponds to milligrammes per litre, is frequently used on the Continent, and is also often employed in this country in expressing the amounts of such substances as ammonia, &c., which occur in waters in infinitely small amounts, though the remainder of the results are expressed in grains per gallon. The second method—that is, parts per 100,000—is the one which was used by the Rivers Pollution Commissioners, and has consequently to some extent official sanction. The third method was formerly much used on the Continent, but seems to be now nearly obsolete. These different methods of statement are easily convertible into one another, if it be borne in mind that the gallon of water contains 70,000 grains; it is obvious that a sum in simple proportion will convert any one form of expression into any of the other forms. For instance, if we have a water which contains 14 grains per

¹ To be consistent with modern chemical science, the constituents should, in the first place, be stated as positive and negative ions, thus, Ca , SO_4 , &c.

gallon of calcium oxide, this amount expressed in parts per 100,000 may be obtained as follows :—

$$70,000 \quad : \quad 100,000 \quad :: \quad 14 \quad : \quad 20$$

or, more shortly, by dividing the number of grains per gallon by 0.7 : thus $14 \div 0.7 = 20$. Similarly, parts per 100,000 are converted into grains per gallon thus—

$$100,000 \quad : \quad 70,000 \quad :: \quad 20 \quad : \quad 14$$

or, shortly, by multiplying by 0.7 : thus, $20 \times 0.7 = 14$.

Grains per gallon are converted into parts per million by dividing by 0.07 : thus, 14 grains per gallon divided by 0.07 equal 200 parts per million ; for the converse process multiply the parts per million by 0.07 : thus, 200 parts per gallon $\times 0.07 = 14$ grains per gallon.

Attempts have been made at various times to put an end to the statement of results in so many different ways, and to induce chemists to employ one uniform plan, but so far these have been unsuccessful.

As has been observed before, the bases and acids are determined separately in every water analysis, but it is customary to append to each report, in addition to the bare statement of the results obtained, another which represents the way in which the bases and acids are probably combined as salts in the water. This statement is, however, of an entirely arbitrary nature, because the way in which the bases and acids are combined in a water is not known with absolute exactitude.

It has long been recognised that it is impossible to imitate artificially a mineral water ; such a water may be analysed with all possible care, and its ingredients exactly ascertained. But if these are added to distilled water in the exact proportions found, the artificial water produced in this way will differ, as Professor Liebrich points out, “from the natural one in taste and value ; and this difference is not easy to explain.” As being in strict accordance with this view, the results of experiments conducted at a brewery in Berlin may be cited, where it was attempted to produce beer having the qualities of the renowned Munich beer. The water was artificially prepared so as to resemble the Munich water as closely as possible, and all the other materials, with the exception of the water, were imported from the Bavarian capital. The quality of the beer produced, however, did not reach the Munich standard.

The statement of the results of a water analysis, in which the constituents are given in their probable state of combination, is convenient, and enables an opinion to be more easily formed as to the general characteristics of the water ; than one which gives a mere statement of the various bases and acids it contains.

Waters considered in Respect to the Geological Formations in which they occur.—The composition of the various rocks, strata, &c., which water meets with, either in percolating through the soil or in flowing over its surface, differs widely. The salts found in a particular water depend

upon the composition of the various strata with which it comes in contact, and these will differ as the geological formation of the district varies. It is impossible to say with absolute certainty what these strata will be in any particular district, because, as a rule, the information we possess is not sufficiently minute as to what the geological formation of any particular district is, for only the broad features have been laid down by geologists, and these are usually insufficient for this purpose. Thus a hard selenitic (calcium sulphate) water may be found occasionally in the middle of a sandy district which yields in other places a soft water.

Hardness of Water.—This term, which is in every-day use, is in reality a rough expression of the comparative soap-destroying power which a water exerts before it permits the soap to exercise its well-known detergent action. Every one is aware of the fact that, when soap is used with a very hard water for the purpose of ablution, a certain amount of soap has to be dissolved before a lather can be formed; whereas, when a very soft water is employed, a lather is obtained at once: the formation of a lather serves to indicate the point at which the cleansing action of the soap commences. Dr. Clark very ingeniously founded a process for the comparative estimation of the hardness of waters on this principle. In this an alcoholic solution of soap of a definite strength is added little by little to a measured quantity of the water placed in a stoppered bottle. After each addition of soap solution the water is vigorously shaken, and so soon as a lather forms on its surface, which is permanent for two minutes, the quantity of soap solution is read off, and from the amount used in this way the hardness of the water is expressed in degrees. Soap is a compound of an alkali, such as soda, with one or more of the fatty acids; and when its solution is added to a water containing calcium or magnesium salts, decomposition ensues, and the earthy bases combine with the fatty acids to form insoluble compounds; it is only after the whole of these bases are precipitated that a lather can be formed. The formation of the first lather indicates that the point has been reached at which all the earthy bases have been removed from solution; consequently, the amount of soap solution used to reach this point forms the basis for an estimation of their quantity.

A sample of water is tested in this way both before and after boiling. Obviously a water containing the acid carbonates of lime and magnesia will show a less degree of hardness after boiling (p. 410). The amount removed in this way is termed the “temporary hardness,” in contradistinction to that which depends upon the presence of the sulphates and chlorides of the earthy bases, and which, not being removable by boiling, is termed “permanent hardness.”

Since the term “hardness of a water,” when applied according to its commonly accepted meaning to a class of waters very often used for brewing purposes, conveys an entirely erroneous impression as to the character of such waters, this term, if applied at all to brewing waters, should be strictly limited to permanent hardness. As Clark’s test is but a rough one, it is

much better to form an opinion of the hardness of a water from the results of an actual analysis, by means of which it is possible to learn exactly what proportion of the calcium and magnesium salts will be precipitated on boiling and what proportion will remain in solution.

Consideration of Brewing Waters with Reference to their Mineral Constituents.—Since water is one of the most important substances used in brewing, it is one of the first and most indispensable conditions that every brewery should possess an abundant and never-failing supply of suitable water. The most usual sources of brewing water in this country are wells and springs, but occasionally lake or river water is employed; and rain water, if collected under proper conditions, may also be employed. As a proper supply of water is of primary importance in brewing operations, before the erection of a brewery is commenced in any particular locality, it is absolutely necessary to ascertain whether the district is capable of affording a sufficient amount of water of the requisite quality. Though this proposition may appear to be self-evident, yet instances are not unknown where breweries have been erected without an investigation of this kind having been made, and where considerable difficulties have subsequently arisen from the oversight.

The waters obtained from the different sources just mentioned differ considerably as to the amount of organic and inorganic constituents which they contain. Some of them are eminently adapted in their natural state for the production of one particular class of ale, and can be modified by artificial treatment so as to become fitted for the production of ales of all classes; others do not lend themselves readily to such treatment; whilst others, either from the amount of organic pollution to which they have been subjected, or from the quantity or quality of the inorganic salts which they contain, are entirely unfitted to enter into the composition of beer, though they may be employed for refrigerating purposes.

It has been found that the waters best adapted for brewing purposes are those which contain inorganic salts in quantities which should not vary beyond certain limits. In all public water-supplies for domestic purposes, the quantity of inorganic constituents demanded by hygienic considerations is much less than that permissible or, as a rule, advisable for brewing purposes; consequently, waters of this class are not generally fitted for the production of all the classes of ales without further modification; but in all cases these waters may be so treated artificially as to render them suitable for use in the brewery. The waters chiefly used for brewing purposes are derived from wells or springs, though rain, lake or river water may also be used for this purpose, provided it possess the requisite purity, organically and inorganically. The waters derived from springs, wells, &c., as met with in various parts of the country, differ widely in the quantity and nature of the inorganic constituents which they contain. Of the various salts generally met with in well and spring water, some, such as calcium and magnesium sulphates, sodium, magnesium, and calcium chlorides, exercise a beneficial influence

when not present in too large quantities; whilst others, such as sodium sulphate and carbonate, when occurring in any considerable quantity, are prejudicial. Naturally, water for brewing purposes must not contain any of the salts of the poisonous metals, and the salts of iron, when present in anything but mere traces, are detrimental.

Experience has shown that the waters of some districts are particularly adapted for the production of ales of one particular character, whilst they are unsuitable for the production of those of another class. Thus the waters found at Burton-on-Trent have been long renowned for yielding excellent pale ales, whilst those of Dublin have been equally famed for producing black beers. In other districts waters are found which are admirably adapted for the production of mild ales. When investigations were made into the chemical constitution of these various waters, it was found that the difference in their behaviour in brewing operations bore a constant relation to their chemical composition. Thus the waters of Burton were found to contain large quantities of sulphate of lime, and to the presence of this salt their excellence in the production of that particular class of ales for which this district has been so long famed is undoubtedly to be attributed. The Dublin waters, on the other hand, were found to contain calcium sulphate in small quantity, but fairly large amounts of calcium and magnesium acid carbonates; and, since these latter salts are almost completely precipitated on boiling, the water as it is actually used in brewing contains but two or three grains of solid matter per gallon. The waters which experience has shown to yield the best mild ales also show a marked difference in composition from the other two. In these, calcium sulphate only occurs in quantities of from 5 to 7 grains per gallon, whilst sodium chloride predominates. These important facts having been established, it occurred to chemists that a water inorganically unsuitable to the production of a particular class of ale might be so modified in its chemical composition as to be rendered fit for the production of another class of ale. It was found that some waters could be so treated, and thus arose the various systems for treating water chemically which have been practised to such a large extent during recent years, and which have been followed with very advantageous results. Thus the London waters have been long noted for their adaptability for the production of black beers, and for their unfitness for yielding pale ales; so much was this the case, that formerly it was the custom for London brewers either to purchase their pale ales from Burton firms or to establish a branch brewery at the latter place. By chemically treating London waters, it is now found possible to brew pale ales which are able to compete with those produced at Burton.

Waters Adapted for Producing Pale Ales.—As has just been observed, the waters most suitable for the production of pale ales are those which contain calcium sulphate in fairly large quantity. Of these the Burton waters may be taken as typical examples. The following are the results of an analysis¹ of the water from a deep well, situated in that town:—

¹ E. Brown, "The Geology and the Mineral Waters of Burton-on-Trent," p. 23.

TYPE I.

	Grains per Gallon.		Grains per Gallon.
Silica	0.49	Sodium chloride	3.90
Alumina	0.49	Potassium sulphate	1.59
Iron oxide	trace	Sodium nitrate	1.97
Lime	36.33	Sodium sulphate	10.21
Magnesia	10.15	Calcium sulphate	77.87
Soda	7.25	Calcium carbonate	7.62
Potash	0.86	Magnesium carbonate	21.31
Chlorine	2.37	Silica and alumina	0.98
Sulphuric anhydride	52.29		
Nitric anhydride	1.25		
Carbonic anhydride	14.50		

In this and similar waters the amount of calcium sulphate is exceedingly high, whilst a fair amount of calcium and magnesium carbonates are also present; the chlorides are very small in quantity. The amount of calcium sulphate in this particular water is undoubtedly very large, and most probably all the possible beneficial effect to be derived from the presence of calcium sulphate in a brewing water may be obtained with from 40 to 50 grains per gallon of that salt.

Waters Suitable for Black Beers.—As a contrast to this class of waters, an analysis of one of the Dublin well waters is appended:—

TYPE II.

	Grains per Gallon.		Grains per Gallon.
Silica	0.26	Sodium chloride	1.83
Iron oxide and alumina	0.24	Calcium sulphate	4.45
Lime	9.79	Calcium carbonate	14.21
Magnesia	0.43	Magnesium carbonate	0.90
Soda	0.97	Iron oxide and alumina	0.24
Chlorine	1.11	Silica	0.26
Sulphuric anhydride	2.62		
Carbonic anhydride	6.72		

Waters of this class are distinguished by the small quantity of calcium sulphate and all the other constituents, with the exception of calcium carbonate, which they contain. This last salt is almost entirely removed on boiling such a water,

Waters Fitted for Mild Ales.—The following is the analysis of a water adapted for the production of mild ales:—

TYPE III.

	Grains per Gallon.		Grains per Gallon.
Silica	0.22	Sodium chloride	35.14
Iron oxide and alumina	0.24	Calcium chloride	3.88
Lime	13.13	Calcium sulphate	6.23
Magnesia	1.91	Calcium carbonate	16.37
Soda	18.62	Magnesium carbonate	4.01
Chlorine	23.81	Iron oxide and alumina	0.24
Sulphuric anhydride	3.67	Silica	0.22
Carbonic anhydride	9.34		

The essential characteristic of this class of waters is the high amount of chlorides and the comparatively small amount of calcium sulphate which they contain.

Artificial Treatment of Brewing Waters.—From the above generalisations of the inorganic constitution of those waters which have been found by experience to be the best fitted for the production of the different classes of ale, it is obvious that no single water possesses the qualifications necessary for producing every class of beer. Fortunately, the knowledge acquired during the past few years has shown that it is possible so to modify the inorganic constitution of many waters that this important result may be attained. Some waters do not lend themselves so readily to this treatment, and there still remain others which it is absolutely impossible to convert into good brewing waters. The waters from the chalk, which are very frequently met with, are highly valued for brewing purposes.

The following is an analysis of one of these :

	Grains per Gallon.		Grains per Gallon.
Calcium carbonate . . .	17.92	Calcium chloride . . .	0.21
Magnesium carbonate . . .	0.49	Sodium chloride . . .	0.84
Calcium sulphate . . .	0.07	Magnesium nitrate . . .	1.05
Potassium sulphate . . .	0.56	Silica	1.12

Such a water as this, without any other treatment than boiling, is eminently fitted for the production of black beers, since, when boiled, the carbonates are almost completely precipitated, and very little solid matter of any kind remains in solution.

To convert such a water into one suitable for the production of pale ales, an addition of those salts in which the water is deficient must be made, and its inorganic constitution brought more into agreement with that of a water of Type I., of which an analysis has just been given. This will be secured by the addition of the following salts, in quantities equivalent to the following amounts of the respective anhydrous salts in grains per gallon :

Calcium sulphate	50 grains.
Magnesium sulphate	6 grains.
Calcium chloride	10 grains.

These salts, as met with in commerce, always contain definite amounts of water of crystallisation, hence in all calculations this must be allowed for. The amounts of the anhydrous salts given above, sufficient for one barrel of water, are worked out in the following statement, in which their water of crystallisation is taken into account. It is arranged in a convenient manner for everyday use :

Calcium sulphate (gypsum, $\text{CaSO}_4 + 2\text{H}_2\text{O}$) . . .	5½ ounces.
Magnesium sulphate (Epsom salts, $\text{MgSO}_4 + 7\text{H}_2\text{O}$) . .	1 ounce.
Calcium chloride (saturated solution)	1 fluid ounce.

The gypsum, which should be in the state of a fine powder, is intimately stirred into sufficient water to yield a mixture of the consistence of thin

whitewash; the remainder of the ingredients are added to this, and the whole mixed with the water of the hot-liquor tank, when it is in a state of lively ebullition. If the water in the tank is not boiling, the contents of the hot-liquor back should be well roused from time to time, in order to secure the solution of the calcium sulphate, which is by no means a very soluble salt. Obviously the whole of the water used for the brew—mash and sparge liquor—should be similarly treated. Calcium chloride ($\text{CaCl}_2 + 6\text{H}_2\text{O}$) is such an extremely deliquescent salt that it is difficult to handle it in the solid state. Consequently, it is much better to obtain this substance in the form of a saturated solution, which has a specific gravity of 1.380 and contains about 63 per cent. of the anhydrous salt.¹

In order to convert the original water into one suitable for producing mild ales, an addition should be made per gallon equivalent to the amounts of the following salts in their anhydrous condition:

Calcium sulphate	5 grains.
Magnesium chloride	5 grains.
Sodium chloride	30 grains.

This result will be obtained by adding the following quantities of the commercial salts to each barrel of brewing liquor:

Calcium sulphate	$\frac{3}{4}$ ounce.
Calcium chloride (saturated solution)	$\frac{1}{2}$ fluid ounce.
Magnesium chloride (45 per cent. solution)	2 fluid ounces.
Sodium chloride (common salt)	2 $\frac{1}{2}$ ounces

As magnesium chloride, like calcium chloride, is an exceedingly deliquescent salt, it is best kept in the form of a 45 per cent. solution, made by placing 4 $\frac{1}{2}$ lb. of the chloride in a vessel, adding four pints of water, and, when the salt is dissolved, making the quantity up to one gallon. The specific gravity of such a solution will be about 1.160. One fluid ounce introduces 7.5 grains per gallon of anhydrous magnesium chloride.

Another method of securing a very similar result is the addition of three-quarters of an ounce of calcium sulphate and three ounces of Kainit² to

¹ The addition of one fluid ounce per barrel of this solution introduces 10 grains per gallon of anhydrous calcium chloride.

² Kainit is obtained from mines sunk in a very peculiar geological formation which exists at Stassfurt in Germany, and which is supposed to have been formed by the evaporation of such an inland sea as the Dead Sea. The lowest bed consists of an enormous mass of rock salt, interspersed with thin veins of gypsum. Next comes a layer of Polyhallite ($2\text{CaSO}_4 + \text{MgSO}_4 + \text{K}_2\text{SO}_4 + \text{H}_2\text{O}$), then one of Kieserite (KCl 11.8 per cent., MgSO_4 21.5 per cent., MgCl_2 17.2 per cent., NaCl 26.7 per cent., CaSO_4 8 per cent., H_2O 1.3 per cent.), then Carnallite (KCl 15.5 per cent., MgCl_2 12.4 per cent., MgSO_4 14.5 per cent., NaCl 22.5 per cent., CaSO_4 1.9 per cent., H_2O 26.1 per cent.). On the top of this last bed is found the Kainit, above which there is found relatively small quantities of Sylvinit (KCl 30.55 per cent., K_2SO_4 6.97 per cent., MgSO_4 4.80 per cent., MgCl_2 2.54 per cent., NaCl 46.05 per cent., CaSO_4 1.80 per cent.; H_2O , &c., 7.29 per cent.). The way in which these deposits occur points strongly to their various components having been separated from an aqueous solution by slow evaporation, the less soluble salts being the first to be deposited. Most of these beds, with the exception of the rock salt, are now largely mined for manurial purposes, the large quantities of potassium salts which they contain rendering them very valuable manures.

every barrel of mash liquor. Kainit is a mineral substance which in its pure state contains one molecule of potassium sulphate, one of magnesium sulphate, and one of magnesium chloride, together with six molecules of water ($K_2SO_4 + MgSO_4 + MgCl_2 + 6H_2O$). But as met with in commerce it is never pure, being largely contaminated with sodium chloride. Its average composition, as given by Maercker,¹ is as follows:

	Per cent.
Potassium sulphate	21.3
Magnesium sulphate	14.5
Magnesium chloride	12.4
Potassium chloride	2.0
Sodium chloride	34.6
Calcium sulphate	1.7
Clay	0.8
Water	12.7
	<hr/> 100.0

We now come to another and numerous class of waters, which require the addition of various salts not only to supply the natural deficiency of the waters in certain saline constituents, but also to remove by double decomposition salts which are in themselves detrimental. For instance, suppose we have a water containing 10 to 15 grains of calcium sulphate and 5 or 6 grains of magnesium sulphate per gallon, which is in other respects of fairly pure quality, and that we wish to produce black beers with such a water. To do this successfully the calcium and magnesium sulphates must be removed, and this can be effected by adding sodium or potassium carbonate, or a mixture of these, in proportions equivalent to the respective amounts of the sulphates we wish to displace. On boiling such a water, after this addition, double decomposition ensues sodium or potassium sulphate being formed, together with calcium and magnesium carbonates, and these latter, since they are nearly insoluble, are precipitated and removed. Obviously, by such a method we produce a considerable amount of sodium or potassium sulphate, or of a mixture of the two salts, and these salts, especially sodium sulphate, are not very desirable ingredients in a brewing water, as they are apt to extract a somewhat coarse flavour from the hops. Still, in the brewing of black beers this is not a matter of such importance as would be the case in the production of a pale or mild ale. The equivalent of anhydrous calcium sulphate is 136; that of crystallised sodium carbonate ($Na_2CO_3 + 10H_2O$), 286; and that of crystallised potassium carbonate ($K_2CO_3 + 2H_2O$), 174. Consequently, each grain of anhydrous calcium sulphate present in a water will require for its decomposition 2.13 grains of sodium carbonate, or 1.28 grains of potassium carbonate; similarly, each grain of anhydrous magnesium sulphate (equivalent 120) will require 2.38 grains of sodium carbonate, or 1.45 grains of potassium carbonate.

Obviously, the calcium sulphate of such a water as that of Type I. might be removed in this manner; but, in such a case, the large amount of sodium

¹ *Die Kalidungung*, p. 5.

or potassium sulphate formed would render the water unfitted for brewing any class of ale.

Many waters are met with that contain small quantities of calcium and magnesium in the form of carbonates only, but which contain the carbonates and sulphates of the alkalies in considerable proportion. Of such a nature are the waters found in the strata beneath London, which are derived from the green-sand below the chalk. It is from these waters that the black beers for which London has so long been celebrated are brewed. The following are the results of an analysis of one of these :

	Grains per Gallon.
Sodium carbonate	11.13
Sodium chloride	9.88
Sodium sulphate	41.3
Calcium carbonate	6.75
Magnesium carbonate	1.85
Silica and alumina (including traces of calcium sulphate)	0.39
Iron oxide	0.49

Such waters as these in their natural state are eminently fitted for the production of black beers, but they require considerable treatment before being used for brewing ales. This treatment consists in the addition of substances which convert the salts prejudicial to ale-brewing (the sodium carbonate and sulphate) into compounds of a harmless nature. This object is best effected by the addition of calcium chloride in proper proportion. Double decomposition then ensues on boiling the water: the carbon dioxide of the sodium carbonate goes over to the calcium of the calcium chloride to form insoluble calcium carbonate; whilst the chlorine goes over to the sodium and forms sodium chloride, which remains in solution. The sulphuric acid of the sodium salt goes over to the calcium of the calcium chloride to form calcium sulphate, whilst its sodium unites with the chlorine to form sodium chloride, both which salts remain in solution. Such waters as these may be easily converted into waters well adapted for the production of mild ales, and this would be effected by the addition per barrel of $1\frac{3}{4}$ ounces of saturated solution of calcium chloride. If, however, pale ales are to be produced from one of these waters, a further addition will have to be made; this will be the addition of sufficient calcium sulphate to bring the water up to the standard of Type I., and in this case the quantity to be added would be about $5\frac{1}{4}$ ounces per barrel. Obviously a water treated in this manner would contain an amount of sodium chloride much larger than the typical class of waters best fitted for the production of pale ales; hence an ale produced from such a water after artificial treatment is never equal to one made with real Burton water. Owing to the large amount of sodium chloride which it would contain after treatment, an ale produced from such a water could never have the cleanness of palate and delicacy of flavour possessed by one brewed with the natural water. Much of the "running ales" brewed in London and its

neighbourhood, which are of an exceedingly satisfactory nature, are produced with this class of water in its natural state.

Waters Entirely Unfitted for Brewing Purposes.—Waters are occasionally met with which, owing to the excessive amount of either one or several of the mineral constituents that they contain, are entirely unfitted for brewing purposes. For instance, waters are sometimes found containing over 100 grains per gallon of calcium sulphate. Such waters interfere so seriously with the course of fermentation that it is impossible to produce a satisfactory ale with them. In the case of a water of this kind, which is in other respects a suitable one for brewing purposes, the only way is to dilute it with a purer water, such as that supplied for domestic purposes. Waters are also occasionally met with which contain enormous quantities of sodium chloride, amounting to as much as 160 grains per gallon. These, likewise, are entirely unfitted for brewing purposes, since they prejudicially affect the fermentations, causing rapid weakening of the yeast. Perhaps 50 grains per gallon may be put down as the largest amount of sodium chloride permissible in a brewing water; the only way out of the difficulty with such a water is dilution with one from a purer source.

Removal of Iron from Brewing Waters.—Occasionally salts of iron occur in water in such quantities as to demand their removal. In a water which contains a fair amount of the calcium and magnesium carbonates, simply boiling the water effects the removal of the iron. The calcium or magnesium combines with the acid of the iron salt, iron carbonate being formed, which, at the temperature of boiling water, is quickly converted into insoluble iron oxide, and is precipitated along with the calcium and magnesium carbonates. Where these bodies do not exist in a water, the removal of the iron is best effected by the addition of sodium carbonate in quantity rather more than is sufficient to decompose the iron salt present, and after the water has been well boiled, adding calcium chloride in a rather larger quantity than is sufficient to decompose the excess of sodium carbonate. In the report of a water analysis the iron is always returned as ferric oxide. An iron salt containing one grain of ferric oxide (Fe_2O_3) requires for its decomposition 1.79 grain of crystallised sodium carbonate; and for the decomposition of one grain of crystallised sodium carbonate 0.4 grain of calcium chloride is necessary. Thus, in a water shown by analysis to contain 2.26 grains per gallon of iron oxide, 4.05 grains per gallon of crystallised sodium carbonate will be required to precipitate the iron salt present. To effect this object in actual practice, it is best to add a little more than the theoretical quantity—say, in this case, 6 grains per gallon, which would leave an excess per gallon of 1.95 grain of sodium carbonate. For the decomposition of this quantity of sodium carbonate $1.95 \times 0.4 = 0.78$ grain of anhydrous calcium chloride would be required, and as the addition of $\frac{1}{10}$ th of a fluid ounce (20 fluid ounces = 1 pint) of a saturated solution of this salt to each barrel of water would be equal to the addition of about one grain per gallon, the object in view would be effected, and a slight excess of calcium chloride left.

Influence of Boiling upon Certain Waters.—The chemical composition of many waters is considerably modified when they are boiled. Waters which contain the acid carbonates of lime and magnesia suffer great alteration on boiling. The second molecule of carbonic acid, which is somewhat loosely attached, becomes liberated, and, as a consequence, the calcium and magnesium carbonates, being insoluble bodies, are precipitated thus :

This reaction takes place gradually, and about half an hour's vigorous boiling is usually required to ensure the fairly complete elimination of the carbonates.

Many natural waters are met with which contain calcium and magnesium sulphates in solution along with acid sodium carbonate. The composition of these is considerably effected by boiling; double decomposition ensues, one molecule of the carbonic acid of the sodium being liberated, the other passing over to the earthy bases, whilst the sulphuric acid from these combines with the sodium, thus :

Consideration of Water with Reference to its Organic Constitution.—Experience has long shown that the presence of any considerable quantity of organic matter in a water used for brewing purposes is highly undesirable; beers produced from such contaminated waters show a great tendency, especially marked in hot weather, to become “fretty,” go turbid, turn sour, and refuse to be clarified in the process of fining. The sources of the organic matter in water are manifold; rivers are often polluted with the refuse of manufactures, such as tanneries, paper mills, &c. &c.; frequently they are defiled by the sewage of towns. Surface water is often contaminated by flowing over arable ground on which manure is spread regularly. Wells in and about towns are often rendered organically impure by the drainage from cesspools, liquid portions of manure from stables, &c. Waters collected from uncultivated land frequently contain large quantities of organic matter of vegetable origin (peaty waters).

The organic bodies which contaminate a water cannot, like the inorganic ones, be directly isolated; consequently their quantity cannot be determined directly. We are therefore obliged to be content with a determination of one or more of the constituents of the organic matter, and to deduce from these the amount of contamination which any particular sample of water has been subjected to. Several of the processes that have been proposed for this purpose will be afterwards described.

In forming a conclusion from the data afforded by the results of a water analysis, it may be useful to bear in mind the following facts. Urine, which contains nearly 1 per cent. of sodium chloride, is one of the most frequent sources of pollution; consequently, a water which contains a much larger amount

of this salt than other waters occurring in the same district, and which are known to be uncontaminated by sewage, is to be regarded with suspicion. A fairly large amount of a highly nitrogenous substance called "urea" is also present in urine, and this, under the influence of certain bacteria, is rapidly transformed into ammonium carbonate; consequently, waters directly polluted by urine contain considerable amounts of free ammonia as well as chlorides. The drainage from sewers, middens, and cesspools, are rich in both chlorine and ammonia. Ammonia is also one of the chief compounds derived from the decomposition of animal organic matter; hence its presence in the water of a shallow well often points to recent pollution with organic matter of animal origin. Rain water as it descends through the atmosphere dissolves a fairly large amount of ammonia; in districts remote from towns the average amount of this substance found in rain water is 0.49 part per million parts of water; in towns the quantity may reach 2.10 parts per million. Nitric acid is also found in a small quantity in rain water, in country places about 0.12 grain per gallon. Much chlorine is often present in rain water falling near the sea; as much as 3 grains per gallon of chlorine has been found in such a water. The washings of solid excreta contain little chlorine, but much organic matter, and also phosphoric acid.

Effect of Filtration through, and Drainage over, the Soil on the Organic Constituents of Water.—Filtration through the soil is the great natural means for the purification of water, and its beneficial effects are most marked in soils of a loose, porous nature, especially when these are well aerated. In passing through them, the ammonia which is present in the water, whether derived from the atmosphere or from the putrefaction of organic matter, is rapidly oxidised into nitrous and nitric acids by certain bacteria which exist in every soil; and these acids, immediately they are formed, combine with one or other of the alkalis or alkaline earths present in the soil to form the corresponding salts. It has been found that 97 per cent. of the nitrogen of the London sewage is converted in this way into nitric acid by slowly traversing a stratum of gravelly soil 5 feet thick. A water containing a large quantity of nitric acid has almost certainly been contaminated, at some portion of its history, with organic matter of animal origin. Vegetable matters, under like circumstances, yield either no nitric acid or mere traces. As the presence of nitric acid (combined as nitrates) in any quantity less than 10 grains per gallon may be permitted in a water used for brewing purposes, the presence of nitrates to this amount in the water of a deep well is of no importance, since such a water must have passed through an immense thickness of soil, rock, &c., before being finally collected. When, however, nitric acid is present in large quantity in the water of a shallow well, the matter stands very differently, and especially when this acid is accompanied by nitrous acid. Here the filtering strata are of a comparatively small thickness, and though they may be sufficient at most times to convert into nitric acid and render innocuous all the organic matter originally present in the water, yet there is always the danger that the filtering power of such a soil may become over-

taxed, and then organic matter in a state of putrefaction will obtain access to the well. The soil itself has also the remarkable property of absorbing and retaining several substances, such as ammonia, potash, phosphoric acid, &c., which the plants growing upon its surface remove for their nourishment; hence, water which is obtained by surface drainage from uncultivated tracts of land, such as moors, is found to be almost destitute of these bodies, though ammonia and nitric acid are invariably contained in the rain which falls on the soil. When the water is collected from cultivated tracts of land, the influence of manuring becomes evident by the appearance of ammonia and nitric acid in the water.

Ammonia is often contained in fairly large amounts in the water of deep wells, especially in those which are sunk through the London clay into the chalk. In this case it is derived from the nitrates present in the water, and is of even less importance than the nitrates from which it is derived, since it is still more remote from the original organic contamination. Waters derived from deep wells often contain large quantities of nitric acid as nitrates, these may occasionally amount to 4 or 5 grains per gallon.

The Combustion Process—Sir Edward Frankland.—In this process a quantity of water is first boiled with sulphurous acid, to remove nitric and nitrous acids and carbon dioxide, a trace of ferric chloride being also added to ensure the destruction of the nitrates. The water is then evaporated to dryness, certain precautions being taken so as to ensure that no impurity is taken up by the water during this part of the process. The dry residue is then removed from the dish, mixed with cupric oxide, and placed in a combustion tube. The tube is then partially filled with cupric oxide, a cylinder of copper gauze being placed in front, and afterwards another layer of cupric oxide. The tube and its contents are now placed in the combustion furnace, connected with a Sprengel air-pump, and the air completely exhausted. Heat is gradually applied to the tube, and the organic matter in the water-residue is burnt up by the combined agency of heat and the oxygen liberated from the cupric oxide, its carbon being given off as carbon dioxide, its hydrogen as water, and its nitrogen as nitrogen and nitric oxide. The gases as they escape are collected in a jar over mercury, and, when the combustion is finished, are transferred to a graduated tube, and their respective quantities measured according to the ordinary methods. From the measurements thus obtained the respective weights of the nitrogen and the carbon dioxide are calculated. These are then calculated into parts per 100,000 parts of water, and expressed in these terms in the statement of the result of the analysis. In addition to this, the free ammonia in the water, the chlorine, the nitrogen of the nitrates and nitrites, as well as the total solids of the water, are estimated and recorded.¹ The following are the results of the analyses of two waters by this process; the first of these is a very pure water, the second is one which has undergone considerable organic contamination:—

¹ For further details of the process, Frankland's "Water Analysis," may be consulted.

Statement of Analysis in Parts per 100,000

Source of Water.	Total Solids.	Organic Carbon.	Organic Nitrogen.	Ratio of C to N.	Ammonia.	Nitrogen as Nitrates and Nitrites.	Total Combined Nitrogen.	Previous Sewage Contamination.	Chlorine.
Loch Katrine . .	2.40	0.185	0.022	8.4:1	0.001	0.000	0.023	0.000	0.85
Polluted well . .	77.44	0.294	0.186	1.6:1	0.026	2.130	2.337	21.190	12.40

In forming an opinion of the organic purity or otherwise of a water from the results of an analysis in this form, the absolute amounts of organic carbon and nitrogen are first considered. In a water fitted for domestic purposes the organic carbon should not exceed 0.2, nor the organic nitrogen 0.02 part per 100,000 of water; but in a water employed exclusively for brewing purposes considerably more latitude may be allowed, say four times these amounts.

The ratio existing between the *organic* carbon and nitrogen is next taken into account, and this is supposed to afford considerable evidence as to the kind of pollution the water has been subjected to. When the pollution has been of a vegetable origin the ratio of organic carbon to nitrogen is high, about 8 : 1, or even higher, as may be seen in the analysis of the pure lake water just given. When, on the other hand, the organic matter has been derived from animal sources, the ratio of carbon to nitrogen is relatively low, 3 : 1, or even less, as is exemplified in the case of the polluted well water given in the table.

The original ratio existing between the organic carbon and nitrogen of a water is often considerably modified by processes to which the water is subjected after having received the actual contamination; such, for instance, as exposure to the air, in the case of the water of lakes, streams, or rivers. The organic matter then suffers gradual oxidation, but as its carbon is oxidised away at a more rapid rate than its nitrogen, the ratio previously existing between the two is gradually altered. When an impure water filters through a porous soil, a considerable quantity of the nitrogen of its organic matter is invariably converted into nitrates by the agency of the nitrifying bacteria present in the soil; and in this case, as the carbon is little affected, its quantity remains practically the same. Consequently a disturbance of the C to N ratio takes place, but in the opposite direction to that just described.

In the case of waters from springs and deep wells, owing to the thick strata which these waters pass through, the greater part of the inorganic matter is fully oxidised; the amount of organic carbon remaining ranges from 0.02 to 0.1 per 100,000 parts, the average for deep wells being about 0.6 part carbon and 0.02 part nitrogen. In such waters the C to N ratio may vary from 2 : 1 to 6 : 1, and probably it is here determined more by the extent to which oxidation has taken place than by the original nature of the organic matter.

Previous Sewage or Animal Contamination.—This expression was invented by Frankland as a means of forming some conclusion as to the amount of the organic contamination to which a water had been subjected during some portion of its history, when the contaminating substances had been converted into harmless bodies. It is obtained by adding together the total nitrogen of the nitrates, nitrites, and the ammonia, and deducting from the quantity so obtained an amount of nitrogen equivalent to the average quantity of ammonia found in rain water. The remainder is then converted into parts of sewage, on the assumption that sewage contains 10 parts of nitrogen per 100,000 parts. Thus, if the total nitrogen of the nitrates, nitrites, and ammonia of a polluted water was found to be 2.151 parts per 100,000, from this is deducted 0.032 part nitrogen (the average amount found in rain water), when 2.119 is left, and the “previous sewage contamination” is found by the following proportion sum :

10	:	2.119	::	100,000	:	21,190
N in sewage.		N found.		Parts of sewage.		Previous sewage contamination.

Objections Raised to the Frankland Process.—Many exceptions have been taken to this process, with regard to its want of accuracy, its tediousness, and to the cumbersome and costly apparatus required for its performance.

The Albuminoid Ammonia Process of Wanklyn.—This process is the one most frequently used at present for the determination of the organic matter in water; it is easily performed, and does not require the use of any special apparatus beyond that found in every laboratory. In the determination a certain amount of water is taken (generally half a litre), placed in a retort connected with a Liebig’s condenser, a portion distilled off, and the amount of ammonia contained in the distillate ascertained by Nessler’s reagent; this quantity is registered as free ammonia, as in Frankland’s process. A strongly caustic alkaline solution of potassium permanganate (previously well boiled) is then added to the water remaining in the retort, the whole again submitted to distillation, and the ammonia estimated in successive portions (50 cubic centimetres each) of the distillate. The amounts of ammonia found in the several portions of the distillate are added together, and the quantity thus found returned as albuminoid ammonia. Thus, Wanklyn endeavours to determine the amount of organic nitrogen contained in a water by converting it into ammonia and estimating it as such. In judging a water by this process, Wanklyn states that a water for domestic purposes should not contain more than 0.002 part of free ammonia and 0.005 part of albuminoid ammonia per 100,000 parts. For brewing waters these limits may be considerably exceeded, but, as a general rule, neither the free nor the albuminoid ammonia should, either of them, exceed 0.01 part per 100,000.

Some idea as to the nature of the organic contamination of a water—that is, as to whether it is of animal or vegetable origin—is obtained by noting the rapidity with which the nitrogen is converted into ammonia under the influence of the alkaline permanganate. In those cases where the organic matter is of

animal origin this takes place rapidly, and the first 50 c.c. of the distillate contain nearly the whole of the ammonia; in those where it is of vegetable origin, the successive portions of the distillate contain ammonia in proportions more nearly alike, the second portion of the distillate containing an amount of ammonia but slightly less than the first one, and so on with the third and following portions.

Objections to the Wanklyn Process.—It has been urged against this process that the total amount of the nitrogen of the organic matter of the water is never obtained as ammonia, and this is undoubtedly true. In some comparative experiments made on the same waters by the two processes, Dr. Hill found that the ratio of the nitrogen found by the Frankland method to that of the ammonia yielded by the Wanklyn method varied from 15 : 1 to 4 : 1. In a series of experiments where various nitrogenous organic bodies were submitted to the Wanklyn process, it was found that some of them gave up the whole of their nitrogen as ammonia, others two-thirds, some one-fourth, some very little, and others, amongst which urea is a notable example, yielded none whatever. In spite of this, Dr. Cornelius Fox¹ states that on comparing the details of the results of ninety-nine analyses obtained by both processes, only in one instance was there a distinct conflict of opinion as to quality of the water, and this divergence was readily explicable. Experience has taught that the indications of the Wanklyn process, when taken in conjunction with the other details of a water analysis, are capable of yielding much useful information, and the extensive practical application which the process has met with also points to the same conclusion.

Determination of the Organic Matter in a Water by the Quantity of Oxygen Required to Oxidise it completely.—This process, which was originally invented by Forchhammer, has received various modifications at the hands of other analysts, amongst these the late Dr. Tidy, who proposed the modification that is generally used in this country. A measured quantity of the water is first acidified with sulphuric acid, and a known quantity of potassium permanganate in solution added. The mixture is allowed to stand for several hours (generally three), at the end of which time the amount of undecomposed potassium permanganate remaining in the water is estimated. In most cases a certain amount will have disappeared, having been destroyed in oxidising the organic matter, and this amount, stated in terms of the quantity of oxygen which it would yield up under the conditions of the experiment, is returned in the results of the analysis as "oxygen required to oxidise the organic matter." Its quantity in a water for domestic purposes should not exceed 0.05 part per 100,000 parts, but may probably be allowed to rise as high as 0.25 in a water used for brewing purposes.

Nitric Acid Existing as Nitrates.—There are several methods of estimating nitric acid in water, all of which are equally reliable. There are two ways of stating the quantity of nitric acid found in analysis, as "nitric acid" (really nitric anhydride N_2O_5), or as "nitrogen existing as nitrates."

¹ "Sanitary Examinations of Water, Air, and Food," p. 63.

It must be borne in mind that when stated in the former method the same amount will appear much larger than when stated in the latter way; the ratio between the two is 14 to 54; consequently, nitrogen as nitrates $\times 3.86$ = nitric acid, and nitric acid $\div 3.86$ = nitrogen as nitrates. Pure waters from different sources contain various amounts of nitrogen as nitrates; spring water and river water often contain 0.2 grain per gallon, whilst water from wells in the chalk often contains from 0.6 to 0.7 grain per gallon. In a water for domestic purposes the nitrogen thus combined should not exceed 0.5 grain per gallon; but for brewing waters it may be allowed to rise to 1.5 grain per gallon, and waters containing as much as 2.5 grains per gallon are known which produce excellent beers, but with these latter amounts the water must be organically pure in other respects. Nitrates in excessive amount cause rapid weakening of the yeast.

Nitrogen Existing in a Water as Nitrites.—The nitrogen existing in a water in this condition can be accurately estimated by several processes. Nitrites, when present, should always be looked upon with the greatest suspicion, as, in the vast majority of cases, they indicate that organic matter is obtaining access to the water of a well in quantities beyond those which the adjacent soil, acting in its capacity as a natural filtering-bed, can convert into the less harmful nitrates. If the filtering powers of a soil under such conditions should become a little more taxed, then organic matter in its most objectionable form will obtain entrance to the well. Moreover, it has been shown by Laurent that nitrous acid, when set free from any of its salts by an acid in the fermenting wort, exerts a very prejudicial effect on the yeast, and as all worts contain a certain amount of free acid, there is a great probability that this result will take place in the fermenting vat when a water containing nitrites is employed.

Chlorine Viewed from an Organic Standpoint.—As has been stated before, the presence of chlorides in fairly large amount are, in many cases, decidedly beneficial in a brewing water. When, however, the other indications of the water point to the chlorides being derived from sewage, an excess of these salts must be regarded from an entirely different point of view. Good natural waters contain from 0.7 to 1.5 grain of chlorine per gallon; those from the greensand often contain as much as 5 or 6 grains per gallon; while in the neighbourhood of the sea, well waters may contain still larger amounts of chlorine without its being an indication of organic pollution. One of the best guides as to the significance of the amount of chlorine in a water is to compare it with the quantities contained by waters in the same district which are known to be unpolluted.

Phosphoric Acid.—This acid, which exists in combination with some one or other of the bases present in nearly every water, is as a rule, met with only in traces. Its amount is seldom estimated quantitatively, unless for some special reason; the precipitate produced on the addition of a small quantity of ammonium molybdate and nitric acid to a water residue is noted; and the quantity estimated in this way is returned as "minute traces," "traces," or

“heavy traces.” The presence of phosphoric acid, in anything but the smallest traces, may generally be regarded as an indication of pollution with organic matter of animal origin, since all animal excreta contain an abundance of phosphoric acid.

Behaviour of Water Residues on Ignition.—In every water analysis it is usual to estimate the total solids of the water, in order to obtain a check on the other results of the analysis. For this purpose a measured quantity of the water is evaporated to dryness in a platinum dish over the water-bath, the residue dried in a hot air-bath at 180° C., and weighed. A perfect agreement between the amount thus obtained and the sum of the respective quantities of the bases and acids, determined separately in the analysis, is never obtained; because, during the evaporation of the water, the magnesium chloride partially loses chlorine and becomes converted into a basic salt; the silica displaces some of the carbon dioxide of the carbonates; the last traces of moisture are expelled from the magnesium carbonate with great difficulty; and this salt, though it is recorded in the results of the analysis as the neutral carbonate, is really left in the residue in the form of basic magnesium carbonate. Some of the salts retain their combined water with great tenacity. In addition to these factors more or less organic matter is always present in a water residue, and it is not possible to determine this with any approach to accuracy; nor is any notice taken of the effect which the ammonia compounds may exercise on the other constituents of the water residue. In order to overcome this difficulty, Fresenius¹ directs that all the salts in the water residue shall be converted into sulphates. During the process of ignition after the addition of the sulphuric acid, all the organic matter is destroyed and removed. The separate bases, as obtained in the detailed analysis, when calculated into their respective sulphates, ought to add up to an amount closely agreeing with the quantity obtained by treating the water residue with sulphuric acid in this manner.

It is customary to ignite a water residue, cool, and again weigh, the loss in weight being taken as organic matter. It must be remembered, however, that, under these circumstances, the carbonates lose more or less of their carbon dioxide, and although this may be replaced by treating the residue with ammonium carbonate solution, evaporating to dryness, and then igniting again very gently, even when these precautions are taken, no reliable result can be obtained in this way, for we do not know the exact state in which the magnesium salts are left behind in a water residue after evaporation and ignition; also, a further quantity of carbon dioxide is driven out from some of the carbonates by the silica, and as this latter acid combines with the base, that portion of the base so united is not re-carbonated on treatment with ammonium carbonate solution. Something may be learnt, however, from the behaviour of a water residue under these circumstances. In those cases in which a water is free from, or contains mere traces of, organic matter, the residue when ignited suffers no discoloration; when organic matter

¹ *Anleitung z. Quant. Chem. Anal.*, 6th edition, p. 165.

is present in a somewhat higher amount, a transient browning takes place; this soon passes away, and leaves the residue either white or slightly yellow. When a still higher amount of organic matter is present the residue blackens, and the application of a much higher degree of heat is required to remove the discoloration than in the previous case. When the residue contains much organic matter, intense blackening takes place on the application of heat, which is removed only on prolonged ignition, and even then with the greatest difficulty. Under such circumstances, and when the organic matter is of animal origin, an odour like that of burning feathers may often be detected. In the residues of very bad waters which contain an abundance of nitrates, small sparks are sometimes seen, owing to minute deflagrations taking place between the nitrates and the organic matter present; occasionally red fumes are evolved. At times a smell of sulphurous acid is detected, and this points to the presence of sulphur compounds in the water. According to the late Professor Parkes, "three grains per gallon of either vegetable or animal organic matter cause some blackening, six grains per gallon a good deal, and ten grains per gallon a great amount," in water residues on ignition. The residues of peaty waters often blacken considerably on ignition, but this by itself is an indication of no importance, as the organic matter here is of a vegetable nature.

Heisch's Sugar Test.—This test was originally proposed by the late Mr. Heisch as a means of ascertaining the presence of sewage in water. It is performed by filling a perfectly clean stoppered bottle of about five ounces capacity with the water under examination; and adding thereto one gramme of the purest cane sugar. The stopper having been accurately adjusted so as to exclude atmospheric air, the whole is kept, exposed to the light, at a temperature of 70° F. for several days, the sample being inspected at intervals. In the event of the water being impure, small bodies will be seen floating about in it at the end of twenty-four hours, which, when examined under the microscope, are seen to consist of cells with very brilliant nuclei. These cells afterwards unite into groups which resemble bunches of grapes. Bad waters turn opalescent and milky, and in very bad cases the odour of butyric acid is developed. A similar experiment is conducted with another portion of the water after it has been well boiled, as some waters, especially those which contain carbonates, no longer develop these organisms after they have been boiled; and this is the most important point to the brewer, for he is always able to boil his water before using it. Should no change take place in three days in a water subjected to this test after being boiled, though it may have responded to the test in the unboiled state, such a water may be used with impunity, provided it is in other ways organically pure; but if a marked action shows itself at or before the end of three days, the water should be regarded with suspicion. Much difference of opinion has been expressed by different observers as to the value of this test. Dr. Frankland considers that the formation of these peculiar bodies is merely an indication of the presence of phosphates in a water; Messrs. Matthews and Lott view the matter somewhat

differently. Whilst confirming to a certain extent the views of Frankland, they state that butyric fermentation takes place only in those waters which contain an abundance of phosphates, and in such cases the rest of the results of the analysis invariably show that such waters have been contaminated. Matthews considers that the reason why some waters do not respond to this test after boiling, which they did before, is to be attributed to the precipitation of the phosphates caused by the expulsion of carbon dioxide which takes place on boiling; for the addition of a mere trace of phosphates to such waters again starts the process, and this serves to show that the organisms are not destroyed on boiling. He found that such a substance as egg-albumin, which contains phosphorus in a state of organic combination, mixed with sugar, forms a better food for bacteria than one composed of mineral phosphates and sugar; and that a water to which albumin has been added, and which has been subsequently boiled and filtered, is capable of supporting the growth of bacterial organisms. He considers, therefore, that if a water containing calcium and magnesium carbonates responds to the Heisch test after being boiled, it may be presumed that such a water contains organic matter of an albuminoid nature.

Microscopical Examination of the Sediment of Water.—An examination of this kind, taken in conjunction with the chemical analysis of a water, is often of assistance in forming an opinion as to its value from an organic point of view. For this purpose a fairly large quantity of the water should be allowed to stand for some time (say twenty-four hours) in a covered conical vessel; a portion of the sediment is then removed by means of a pipette,¹ transferred to a glass slip, and examined under the microscope. The deposit generally consists of earthy particles, such as portions of sand, clay, &c., vegetable and animal organisms, portions of decaying vegetable matter, animal matters in a process of decay, such as the portions of the dead bodies of insects. These substances are found in most waters. Amongst the vegetable matters which afford indications of pollution are such substances as the fibres of linen, hemp, cotton, &c.; amongst animal matters such objects as fibres of silk, wool, human hair, hair of other animals, epithelial scales, decaying muscular fibre, fragments of feathers, &c.

The mineral portion of the sediment may be neglected, as it affords no evidence of contamination. Many vegetable and animal organisms are met with in pure waters, some in running streams, others in still water; and their presence, when existing in comparatively small numbers, is of no serious import, though it is always an indication of the existence of more or less organic matter in the water, as evidently such organisms cannot exist without food. Amongst the harmless species may be placed the *Algae* and *Diatomaceae*, *Euglenae*, *Hydrozoa*, and the larger animal forms, such as the water-flea (*Daphnia pulex*), &c. Bacteria when occurring in large quantities, and especially when these belong to many different species, are of ill-omen, and this applies

¹ Or the ingenious micro-filter of W. J. Dibdin may be employed. For description see *The Analyst*, 1896, 21, 3.

also to such organisms as the *Rhizopoda*, *Amœba*, &c. *Vibriones* and *Spirilla* are among those forms of bacterial life which indicate the presence of organic matter in a state of putrefaction. The presence of fungi is also undesirable. But the most objectionable matters are those which point most conclusively to a water having been subjected to direct pollution, such as the presence of the organic substances mentioned in a previous paragraph (see p. 419).¹

The detection of these various objects found in water requires a considerable amount of skill in the use of the microscope, which can only be acquired by long practice. Their examination is, however, an extremely interesting and fascinating study, and one which can be recommended as an excellent means of educating the eye of the observer, and enabling him to form a keen discrimination in the form and size of objects when viewed through the microscope. The water from almost any pond or ditch will be found, especially during the hotter months of the year, to contain microscopic organisms in abundance.

¹ To those interested in this study, "A Guide to the Microscopical Examination of Drinking Water," by Dr. Macdonald (Churchill), which contains engravings of a large number of the organisms and substances usually met with in water, will be found extremely useful.

CHAPTER VII.

BARLEY AND MALTING

Barley.—Though a number of the seeds of the various species of the *Gramineæ* or grasses have been at times used for the purpose of malting, barley has been found, from an experience of many hundreds of years, to be the grain best adapted for yielding a malt, possessing to the fullest extent those properties which are necessary for the production of good beer. It seems also to be the grain most naturally adapted for malting, for it suffers less injury in the process than any other kind of corn. At the same time the seeds of other members of the *Gramineæ* are still malted to a limited extent, for example wheat and oats, the malts produced from them having characteristics which fit them for specific purposes, although, as a rule, they are used in admixture with a relatively large proportion of barley malt.

Barleys are often spoken of as six-rowed and two-rowed; in the first of these varieties there are three rows of spikelets on each side of the ear, each spikelet of which bears a fertile flower. Consequently, when ripe, there are six rows of barley-corns on each ear. In the so-called four-rowed variety (compare p. 428) the middle rows of spikelets on each side of the ear are abortive—that is, produce no seeds—consequently in an ear of this variety there are four rows of barley-corns. In the two-rowed kind only the central rows on each side are fertile; so that only two rows of corns are produced. The corns from the six- and four-rowed varieties may be distinguished by a peculiar curving of the furrow, which characterises all the corns of the four-rowed variety, and also those of the outside rows of the six-rowed barley. The best malting barleys are those grown on light, warm, friable soils; but fine barleys can also be produced on rich, loamy, well-drained soils. On the other hand, good malting barley is rarely, if ever, yielded by stiff, heavy, cold, clayey land.

Until comparatively late years all the malt used in this country for brewing purposes was made from home-grown barley; now much is imported from foreign countries, as, owing to the more regular climatic conditions, the barleys there grown are more uniform in character.

Malting Barley.—The malting barley generally grown throughout the United Kingdom (until recently, almost exclusively) has been the narrow-eared, two-rowed *Hordeum distichum* (see Fig. 73, p. 425) now commonly called

"Chevallier."¹ The Rev. John Chevallier thus describes the origin of this variety: "A labourer (named Andrews), living in a cottage of mine at Debenham in this county (Suffolk), as he passed through a field of barley plucked a few ears, and on his arrival home threw them for his fowls into his garden, and in due time a few of the grains arrived at maturity, and as the ears appeared remarkably fine I determined to try the experiment of cultivating them." According to this, as Messrs. Munro and Beaven point out, the variety was a selection from an already-existing type of grain, common to the district and to the country at large.

The quantity of barley of the Goldthorpe type (*H. zeocritum*; see Fig. 72, p. 425) which is malted has increased for some years, and it must be admitted that this barley often gives excellent results. The paleæ of Goldthorpe barley are somewhat "greasy" in appearance, and do not adhere so closely to the corn as in the Chevallier. Goldthorpe barley is further characterised by a small dimple or transverse furrow in the skin near the extremity of the basal end of the corn. The basal bristle (rachilla) of Goldthorpe barley is invariably covered with long hairs, whilst that of Chevallier has, as a rule, very short hairs, though the Archer variety and some others possess long hairs somewhat resembling those of the Goldthorpe type.²

One sort of Goldthorpe barley was commonly known as Italian or Fluck wheat, another as Sprat, Fan, Peacock or Battledoor barley, and these are described by Lisle, Brown, Low, Sinclair, Loudon, Morton, Wilson and other agricultural authorities.³

Munro and Beaven (*loc. cit.*) state that they are unable to explain how the various forms originated, and seeing that cross fertilisation is a process of extreme difficulty and delicacy to carry out artificially, they are of opinion that it rarely if ever occurs naturally. Several permanent varieties have, however, been produced by artificial cross fertilisation. The glumes which enclose the flower of barley normally open sufficiently to allow the stamens to protrude, although they are frequently caught, so to speak, in the act of escaping by the reclosing glumes. Observation has shown that fertilisation has already taken place before the complete or partial escape of the stamens, and although air-borne pollen grains may be likely enough to gain access to the styles of other florets than those of their parent plants, it is not probable that actual crossing takes place. Munro and Beaven suggest that the difference between the shape of the ear of Fan or Sprat and Chevallier barley respectively, may have arisen after many generations under particular conditions, but that there is no direct evidence that this is the case. Mr. W. Dyson, the original grower of Goldthorpe barley, informed Messrs. Munro and Beaven that the type was started from a single ear found in a field of Chevallier. Whether this ear was

¹ We have adopted Messrs. Munro and Beaven's suggestion to amend the usual spelling "Chevalier" to Chevallier so as to correspond with the name of the original grower.—W. J. S., A.R.L.

² A. J. Brown, *Laboratory Studies for Brewing Students*, p. 11.

³ J. M. H. Munro and E. S. Beaven, *Journ. Roy. Agric. Soc.*, 1900, 6.

a "sport" or was seeded by an accidentally present grain of one of the older wide-eared sorts is a question which cannot be answered. Goldthorpe has grown "true to sort" since its introduction. On the important question of whether the wide-eared barleys are of equal malting value to those of the Chevallier type, Munro and Beaven think that they will not supplant the latter and that they show a greater tendency to deterioration. They state further that whilst all varieties of grain deteriorate under certain conditions the reverse frequently occurs and that speaking generally the quality of Chevallier barley has been fully maintained, if not improved, during recent years. Well-matured Goldthorpes work evenly and freely on the floors, but their special merit is that, in consequence of their having a stouter straw, they stand up under weather conditions which would "lodge" Chevalliers.

Maercker¹ is of opinion that whilst Chevallier is the best variety of barley for soils which, under good climatic conditions, can be relied on to produce a well-matured grain, and although it generally gives better crops on German soils than Goldthorpe, yet the latter variety is more suitable for strong land because it is less susceptible to the ill effects on quality of too good "condition" of soil and does not produce under these conditions so nitrogenous a grain. Maercker finds that a variety of *H. distichum* called "*Hanna*" is best suited for the lightest German soils.

Archer's "Stiff Straw" is a hardy barley of Chevallier type, and according to Munro and Beaven has given good results in the Eastern counties when autumn sown. A two-rowed variety known as "Ouchak" is grown in several Eastern counties. The grains at first sight may be mistaken for six-rowed barley, but on closer examination they will be found to be of quite a distinct shape.

There are two types of six-rowed barley, both of which, when grown in suitable climates, yield the malting material known as "brewing" barley to distinguish it from "Chevallier." *H. vulgare*, the common English winter barley, is grown only for food, whilst the other, *H. hexastichum*, has been reintroduced in some parts of England as a winter barley.

Botany of Barley.—Barley belongs to the genus *Hordeum* of which there are numerous species and varieties. There is a tendency amongst modern botanists to consider all cultivated barleys as belonging to one species, for which, as a whole, the botanical name *Hordeum sativum* has been adopted. Linnæus and the earlier botanists had, however, recognised six species :

<i>Hordeum hexastichum</i>	}	Six-rowed barleys.
<i>Hordeum vulgare</i>		
<i>Hordeum distichum</i>	}	Two-rowed barleys.
<i>Hordeum zeocritum</i>		
<i>Hordeum caeleste</i>	}	Naked barleys.
<i>Hordeum nudum</i>		

Classification of Barley.—Körnicker considers that *H. spontaneum*, a very long, thin-grained, two-rowed barley which grows in the East, to be the

¹ *Journ. Fed. Inst. Brewing*, 1899, 5, 497.

parent form. But E. S. Beaven¹ points out that there is a good deal to be said for the view that original wild species of more than one form were first used as food and afterwards cultivated.

FIG. 70.—*H. Hexastichum*.

- a. Three spikelets *in situ* on the rachis, showing short internodes.
- b. Spike. Median spikelets uppermost, and with lower awns removed.
- c. Spike. Lateral spikelets uppermost, and with lower awns removed.

FIG. 71.—*H. Vulgare*.

- a. Three spikelets *in situ* on the rachis, showing long internodes.
- b. Spike. Median spikelets uppermost.
- c. Spike. Lateral spikelets uppermost.

If an ear or spike of barley be examined it will be found that its primary axis, called the rachis, is divided into internodes, of which there may be any number up to about 40; they each bear three single-flowered spikelets, the

¹ *Journ. Fed. Inst. Brewing*, 1902, 8, 542-594.

three being arranged alternately on either side of the rachis. These three spikelets are attached to the main axis, although a rudimentary secondary axis or rachilla is present to each spikelet which, however, does not function

a b

c d

FIG. 72.—*H. Zeocritum*.

- a, d. Spikelets. Rachis edgewise, showing short internodes.
 b. Var. *zeocritum* (fan barley). Spike converging.
 c. Var. *erectum* (Goldthorpe). Spike parallel.

a b

c d

FIG. 73.—*H. Distichum*.

- a, d. Spikelets. Rachis edgewise, showing long internodes.
 b. Var. *nutans* (Chevallier).
 c. Ouchak barley.

as such and differs in structure in different varieties. Of the three spikelets springing from each internode either all three (*viz.*, the median and the two lateral) or only one (*viz.*, the median) may be fertile. In the former case the barley is said to be six-rowed (Figs. 70 and 71) and in the latter case two-rowed (Figs. 72–75), the superimposed spikelets in each case forming the vertical rows. These descriptions and the matter following (to p. 431) are paraphrased from the paper by E. S. Beaven,¹ whose photographs

¹ *Journ. Inst. Brewing*, 1902, 8, 542–594.

(Figs. 70–79) are here reproduced by the permission of the Council of the Institute of Brewing.

CLASSIFICATION.

Hordeum sativum Persoon (*H. Vulgare*, Körnicke).

SUB-SPECIES OR RACES.

1. Spike of six rows of spikelets, all fertile.
 - A. Wide, with short internodes, *H. hexastichum*, L., Fig. 70.
 - B. Narrow, with long internodes, *H. vulgare*, L. (*H. tetrastichum*, Körnicke). Fig. 71. (Both common.)
2. Spike of six rows of spikelets, all fertile—two median rows normal, four lateral rows diminutive and without awns.
 - A. Wide, with short internodes, *H. intermedium*, Körnicke (var. *Haxtoni*). Fig. 74 a.
 - B. Narrow, with long internodes, *H. intermedium* Körnicke (var. *transiens*). (Both rare.) Fig. 74 b.
3. Spike with two median rows of spikelets fertile and four lateral rows infertile or staminate.
 - A. Wide, with short internodes, *H. zeoeritum*, L. Fig. 72.
 - B. Narrow, with long internodes, *H. distichum*, L. Fig. 73. (Both common).
4. Spike with two median rows of spikelets fertile and four lateral rows rudimentary and with floral organs. *H. decipiens*, Steudel (several Abyssinian varieties.) Fig. 75.

VARIETIES.

The varieties cultivated in different countries differ in the character of the spikelets and their appendages in the following respects :

1. The adherence or non-adherence of the paleæ to the pericarp.
 - a. The common form with adherent paleæ.
 - b. Naked barleys.
2. Colour of (1) the paleæ, (2) the seed (caryopsis). The colour of the seed may be due to the colouring-matter in the pericarp, or in the aleurone cells as pointed out by A. J. Brown.¹ There are furthermore some black barleys which have colouring-matter in the starch-containing cells of the endosperm, whilst they are in a sticky condition, but as the process known as the mellowing proceeds, the colour disappears.
 - a. Yellow or white paleæ and seed.
 - b. Yellow or white paleæ with bluish-grey seed.
 - c. Brown paleæ.
 - d. Black paleæ. The colour of the seed (caryopsis) when naked in the two latter cases also varies.
3. Variation of form of the outer paleæ.
 - a. Normal form with serrated awns. Fig. 76 a.
 - b. With smooth awns.
 - c. Awns replaced by or terminating in three processes, Fig. 76 b, c¹—c⁵, one of which is generally recurved, and frequently bears a rudimentary flower (c¹, c²). The paleæ is then said to be trifurcate.
 - d. Without awns, i.e., the paleæ terminating in a mere point (acuminate) Fig. 76 d.
4. Variation in the glumes.
 - a. The normal form of two narrow spear-shaped glumes, Fig. 77 a, b, c.
 - b. The normal glumes of the median spikelets in the six-rowed sub-species replaced by ovate-lanceolate or boat-shaped bracts, sometimes bearing awns, Fig. 77 d, e, f.
 - c. The glumes of the lateral spikelets so replaced, Fig. 77 g.

¹ *Journ. Fed. Inst. Brewing*, 1900, 6, 480.

- d.* The glumes of all the spikelets so replaced, Fig. 77 *h.* —
5. Variation in the rachilla.
- a.* The rachilla long, with very short hairs (villous), or nearly hairless (glabrous) Fig. 78.
- b.* The rachilla short, and bearing long hairs (hispid), Fig. 79.

a b

c d

FIGS. 74A, 74B.—*H. intermedium*.
Var. *Transiens* Var. *Haxtoni*.

Fig. 74A.

H. intermedium, Kcke. var. *transiens* (wide form).

a. Three spikelets *in situ*, median spikelet uppermost.

b. Spike. Lateral small awnless spikelets uppermost.

Fig. 74B.

H. intermedium, Kcke. var. *Haxtoni* (narrow form).

c, d as *a, b* in Fig. 74A.

a b

FIG. 75.—*H. decipiens*,
Steudel.

a, b as in Fig. 74.

Beaven's classification and nomenclature, as above, differ from those of Körnicke in three particulars. (1) In the common form of six-rowed English barley or Scotch bere (*H. vulgare*), the two lateral rows of spikelets springing from one side of the rachis, either partially or entirely intersect, and overlap

the alternate lateral spikelets which spring from the opposite side of the rachis.¹ This has given rise to the use of the term "four-rowed barley," and the term may be admitted as a common name; but the corresponding botanical term, *H. tetrastichum*, has rather unfortunately, been applied to this sub-species by Körnicke. He transfers the term *H. vulgare* to include all the cultivated barleys (and also *H. spontaneum*, which he supposed the parent

FIG. 76.—Variation in the palea of *Hordeum*.

- a. Normal form.
- b. Short awn terminating in trifid appendage.
- c. 1-3. Trifurcate palea.
- c. 4-5. Trifurcate palea naked grain.
- d. Palea awnless.

form), but English botanists always use the term *H. sativum* as the specific name for all the cultivated barleys, and Beaven deems it desirable therefore to continue to restrict *H. vulgare* to the so-called four-rowed barley, or bere, to which Körnicke gives the new term *tetrastichum*. There is, strictly speaking, no cultivated "four-rowed" barley, as this name would seem to suggest.

(2) Körnicke includes all two-rowed barleys in the sub-species *H. distichum*. Beaven points out, however, that there is just as much reason for ranking the wide short-eared varieties of two-rowed barleys (considered by Linnæus to be a

¹ Very rarely the corresponding *median* spikelet is infertile, so that the spike appears to be "two rowed" towards the apex.

FIG. 77.—Variation in the glumes of *Hordeum*.

- a. Segment of rachis. Glumes *in situ*. Grain removed.
- b. Spikelets of *H. distichum*. Glumes and grain *in situ*.
- c. Spikelets of *H. vulgare*. Glumes and grain *in situ*.
- d. Ovate-lanceolate glume of var. *recens*.
- e. Spikelet of *H. distichum*, var. *Körnickeri*. Glumes lanceolate.
- f. Spikelet of *H. distichum* var. *Braunii*. Glumes of median spikelets lanceolate and awned.
- g. Spikelet of *H. distichum*, var. *Rehmi*. Glumes of all the spikelets awned.
- h. Spikelet of *H. hexastichum*, var. *eurylepis*. Glumes of all the spikelets ovate-lanceolate and awned.

FIG. 78.—Smooth rachilla of Chevallier barley *H. distichum*, *in situ* on the rachis.

FIG. 79.—Bristly rachilla of Goldthorpe barley *in situ* on the grain.

species) as a sub-species distinct from the long narrow-eared, two-rowed varieties, as there is for the closely analogous differentiation of the short and long-eared sub-species *hexastichum* and *vulgare*. This brings the Goldthorpe barleys into the sub-species *H. zeocritum*. They are not as short and wide in the ear as the older form so named; but classification of cultivated species is largely a matter of convenience.

(3) Lastly, at the other end of the scale we have several Abyssinian varieties of two-rowed barley which have hitherto been ignored by English writers, the lateral spikelets of which are quite rudimentary, *i.e.*, reduced to a mere bract, and develop no floral organs. These appear to be even more entitled than the intermediate form before mentioned to rank as a sub-species, and as they are undoubtedly constant in form, Beaven groups them under the name of *H. decipiens*, the name given to them by Steudel, who was one of the first European writers to describe them (*Syn. Gram.*, p. 351).

VARIETIES GROWN IN THE UNITED KINGDOM.

Hordeum hexastichum, commonly known as "six-rowed barley," grown in England only as a winter sort, and mainly as a forage plant, known in France as "Escourgeon." Sometimes imported from Syria; also found in "brewing Chilian."

Hordeum vulgare, known as "four-rowed" or "bere." There are winter and spring sorts in the United Kingdom. Most of the Smyrna and foreign "brewing" barleys also belong to this sub-species.

Hordeum zeocritum is now known here as Goldthorpe or Sprat barley. The earlier and still wider-eared, but more tapering, form, known as "fan" or "peacock" barley, has gone out of general cultivation.

Hordeum distichum is the sub-species to which the Chevallier and all the other narrow-eared two-rowed sorts belong. Ouchak, and many long, thin-grained Eastern barleys, are also in this sub-species.¹

The other sub-species or races are:

Hordeum intermedium (not cultivated anywhere on an agricultural scale), and

Hordeum decipiens, cultivated at present only in Abyssinia.

A large number of the varieties with abnormal forms of spikes are not in general cultivation anywhere, and the majority of them have no agricultural value.

Varieties of Imported Barleys.—The following list, drawn up by Beaven, refers barleys of foreign growth which are in common use in this country

¹ The relative length, size, and shape of the grain, apart from any other feature, important as it is in practice, does not constitute "variation" in a botanical sense. Like colour within certain limits, this feature is only constant so long as climatic and other conditions are unchanged. It is quite conceivable that Ouchak barleys were originally seeded from a Chevallier sort. It is certain that when grown for a few generations in a colder climate the grain becomes much shorter and plumper (Beaven, *loc. cit.*).

to their several botanical sub-species and varieties. Except where a note is appended giving the source of information, Beaven has determined the variety to which the barley is referred by cultivating the grain of the several descriptions on small plots at Warminster.

Chevallier Californian.	<i>H. distichum</i> , with some admixture of <i>H. vulgare</i> , vars. <i>pallidum</i> and <i>coerulescens</i> .
Brewing Californian.	<i>H. vulgare</i> , vars. <i>pallidum</i> and <i>coerulescens</i> .
Chevallier Chilian.	<i>H. distichum</i> with some admixture of <i>H. vulgare</i> .
Brewing Chilian.	<i>H. hexastichum</i> , var. <i>parallelum</i> , and <i>H. vulgare</i> , vars. <i>pallidum</i> and <i>coerulescens</i> .
Mexican.	<i>H. vulgare</i> , vars. <i>pallidum</i> and <i>coerulescens</i> .
Argentine.	<i>H. vulgare</i> .
Morocco, Algeria, and Tunis.	<i>H. vulgare</i> , vars. <i>pallidum</i> and <i>coerulescens</i> .
Tripoli (Ouchak type).	<i>H. distichum</i> .
Gaza.	<i>H. vulgare</i> .
Persian.	<i>H. hexastichum</i> , var. <i>pyramidatum</i> ; <i>H. vulgare</i> , vars. <i>pallidum</i> , <i>nigrum</i> ; <i>H. distichum</i> , vars. <i>nutans</i> , <i>medicum</i> , <i>persicum</i> , <i>nigricans</i> .
Beyrout.	<i>H. hexastichum</i> , var. <i>pyramidatum</i> ; <i>H. vulgare</i> , vars. <i>pallidum</i> and <i>nigrum</i> ; <i>H. distichum</i> , vars. <i>nutans</i> , <i>nigricans</i> .
Smyrna (Yerli).	Mostly <i>H. vulgare</i> , var. <i>pallidum</i> , but frequently mixed with <i>H. distichum</i> .
Ouchak.	Mostly <i>H. distichum</i> (long grained) but mixed with <i>H. vulgare</i> .
Marmora.	<i>H. vulgare</i> var. <i>pallidum</i> .
Black Sea (Azof, Ghenigesk, Bessarabian, Theodosian, Tulcia).	<i>H. vulgare</i> , var. <i>pallidum</i> , generally with some admixture of <i>H. distichum</i> , var. <i>nutans</i> .
Danubian.	Both <i>H. vulgare</i> and <i>H. distichum</i> , and often mixed.
Cyprus.	<i>H. vulgare</i> , var. <i>pallidum</i> .
Spanish.	<i>H. vulgare</i> , vars. <i>pallidum</i> and <i>coerulescens</i> .

Mixtures of two-rowed and six-rowed barleys are, as will be seen, common in most foreign barleys. The relative proportion of each can be approximately ascertained by separating from a sufficient number of corns those in which the furrow is straight from those in which it is curved. The grains of the lateral rows in six-rowed barley have more or less curved furrows, and if the barley is all six-rowed there will be twice as many of these as of grains with straight furrows. The character of the rachilla cannot be relied on to make the separation, because although *vulgare* has generally a rachilla without hairs, and *distichum* of the Ouchak sorts generally have some hairs, this difference is not at all constant.

Mixtures of wide and narrow-eared barley are becoming frequent in native-grown barley. Although, as already stated, Goldthorpes can generally be recognised by a bristly rachilla, whilst true Chevalliers have a nearly smooth one, there are wide-eared barleys with smooth rachilla, and many narrow-eared sorts have a more or less bristly rachilla. The paleæ of the wide-eared barleys are less closely adherent, and the colour is almost always paler. The grain is larger than that of Chevalliers. Threshing machines and drills are

frequently answerable for intermixture which ought to be avoided, especially for seed corn (Beaven, *loc. cit.*).

Mendel's Law of Inheritance.—Barley is rarely, if ever, cross fertilised naturally (see p. 422), and until quite recently little success has attended artificial cross fertilisation experiments. In the year 1901, De Vries, Correns, and Tschermak independently unearthed a remarkable research by Gregor Mendel, which was communicated to the Brünn Society in 1865. By following Mendel's discovery, the result of combining any two parents can be predicted with almost mathematical accuracy, and what is of equal value to the plant breeder, hybrids can be produced as stable as their parents. A valuable paper on this subject was read before the Institute of Brewing by Mr. Biffen of Cambridge.¹

Suppose an ordinary Chevallier barley be crossed with a black variety, when the resulting seeds are sown a black barley is obtained no matter whether the black or white form was used as the male or female parent. The flowers of barley always pollinate themselves, so that the grains of the hybrid plant are produced as the result of fertilisation with the hybrid's own pollen. If these grains are now sown they give rise to Chevallier barleys which are on the average a mixture of black and white corns in the proportion of 3 : 1. No types besides these two are produced, and they both resemble the parents in all external particulars. If now the grains are sown from the white plants it will be found that each plant breeds true to its parent type, but if separate sowings of the black corns are made, it will be found that some give black plants only, and some black and white plants. In this latter case the black plants occur in the ratio of three to one of the white ones. On counting the black plants which breed true to types, and those which give a mixed progeny, it is found that on the average two split up for each one that remains constant. If a further generation is grown as before, the white forms still breed true to their type, those taken from plants in the culture which split up into the two types may or may not reproduce their parent form. If they give a mixed offspring it again consists of black and white individuals in the usual proportion of three to one.

It will be observed that in the above experiment the colour of the hybrid is not a blend of the two parents, but it is just as black as the black parent. The black colour is therefore described as dominant, the white as recessive. Further the plants of the recessive type breed true from the first, and never again split up, whilst some of the plants of the dominant type keep true, others break up and give both parent forms again. The only means of knowing which of the black plants will keep true and which will not is to sow their seeds.

The results may be summed up by means of a simple formula. If the black unit is represented by D, and the white by R, the hybrid will be DR, although to the eye it appears to be pure D. Its progeny after self-fertilisation are in the proportion of 3D to 1R, but on sowing the black corns, it is found that they (the 3D) are not really all alike, for some keep true to type whilst

¹ *Journ. Inst. Brewing*, 1906, 12, 344.

others split up and again give black and white individuals in the proportion of three to one. There are double the number of these latter forms, so that the blacks which were grouped together as 3D are, in reality, 1D (those which keep true to type) and 2DR, those which split up like the plant produced by the process of crossing. The progeny of the original hybrid can then be represented by the formula 1D - 2DR - 1R, though they appear to the eye to be 3D - 1R. This result is, however, only what might be anticipated from the law of chances, for if, as already observed, it makes no difference to the progeny which of the two parents is male and which is female, then the conjugation of DD and RR must be equal to DR, or in other words there must be an equal number of present types and hybrids produced.

Choice of Barley for Malting.—As barley is almost invariably bought in the open market, the purchaser has to rely on certain external signs, the knowledge of which can only be acquired by actual experience in the handling of samples. The following characteristics may be of some little use in starting, but long experience alone will make a person a good judge as to the quality of barley.

Vitality.—One of the most essential conditions of good barley is vitality; obviously, if barley will not grow, it is impossible to convert it into malt. Should, in a particular sample, anything but an extremely small percentage of the grains be incapable of germinating, good malt cannot be made from it, for all ungerminated corns represent merely so much raw barley in the finished malt, and they, moreover, form breeding-grounds for mould during the malting process. Defective vitality may arise from under- or over-ripeness, incipient germination in the ear owing to a wet harvest, death of the germ through heating in the stack, or improper storage, the attacks of vermin and insects, or damage to the grain in the threshing. Barley rapidly loses its vitality in storage, and especially so if stored in a damp condition. Under the best circumstances it should not be used for malting purposes when more than two years old. Barley, especially that which has never been stacked, improves in vitality when stored under favourable conditions for a few months. This is, doubtless, owing to the gradual drying which takes place, for it has been shown by Brown and Morris that barley will not germinate until it has attained a certain degree of dryness.

The most certain test for vitality is to germinate a sample of the barley, but as this takes time, it is of no assistance to the purchaser in the open market. The germination test may be applied in several ways. A hundred to two hundred corns, taken promiscuously from the sample without any picking or choosing, are placed between two layers of flannel on a plate or dish, and the whole covered with water for twenty-four hours. The water is then poured off, the dish and its contents being kept at a temperature of 50° F., when, in from forty to fifty hours, those corns which are capable of germinating will begin to chit—that is, a small protuberance will show itself at that end of the grain where the germ is situated. Contrivances have been invented for effecting experimental

germination, of which Coldewe's germination apparatus (illustrated in Fig. 80) is a good example. It consists of a glass vessel with a constriction about $1\frac{1}{2}$ inch from the top, on which rests a porcelain plate provided with 100 perforations. Into each of these a corn of barley is

FIG. 80.—Coldewe's Germinating Apparatus.

- a. apparatus with water ;
- b. perforated plate ;
- c. felt cover over sand.

inserted, with the germ end downwards. The apparatus is nearly filled with water, the plate inserted, a small quantity of moist sand placed around the barley, and a wooden cover, which has its under side covered with a layer of felt, placed over the sand. A small thermometer attached to the lid serves to indicate the temperature at which the experiment is being conducted. The number of sluggish and dead corns can be seen at a glance, and if the germination be allowed to proceed for a few days, the relative degrees of vitality of the different corns can be readily estimated.

When a large number of samples of barley have to be tested, the apparatus of Aubry¹ is very convenient. It consists of a cubical chamber about 10 inches each way, constructed of sheet metal, and provided with doors in front and behind. On either side of its interior is attached a number of strips of sheet metal about half an inch wide, and fixed at a similar distance from each other ; these serve as supports to the glass plates on which the barley is germinated. The top and bottom of the apparatus are perforated with a few small holes, to admit the quantity of air necessary for the growth of the barley, but not more than this, or too free evaporation will take place. Each of the glass plates is loaded with 500 corns, which are either simply

counted or computed with such an apparatus as that of Westfelt. These are steeped in water for six hours at the ordinary temperature. A piece of damp blotting-paper the size of the plate is first attached to it, then the barley is spread on the paper in an even layer. It is next covered with two similar sheets of blotting-paper, which are afterwards moistened with a damp sponge, when the plate and its contents are placed in the apparatus. The glass plates, which form a series of shelves, are withdrawn periodically for examination, and the manner in which the barley grows noted. The figures thus obtained are converted into percentages by dividing by five.

A convenient apparatus for determining the germinating power of barley is the "Blaber" germinating cupboard. It consists of a cupboard in which the barley is germinated at constant temperature in a moist atmosphere. The barley, after steeping, is placed in small cylindrical metal vessels per-

¹ *Zeit. f. Brau.*, 1885, p. 77.

forated at the top and bottom. Provision is made for the continuous passage of a current of air through the cupboard and hence through the barley.

In examining a sample of barley in the open market, many of the conditions which produce a want of vitality can be determined by an experienced hand, though some of them are so extremely difficult to detect, that they can hardly be discovered in any other way than by the germination test. Corns damaged by vermin or insects can be readily detected, as can also the signs of incipient germination; the acrospire will be slightly developed, and the germ ends of the sprouted grain will have become soft and friable. In extreme cases rootlets protrude. On dividing a corn with a sharp knife through the furrow, and examining the divided germ with the aid of a magnifying glass, this should have, according to Stopes, "a juicy, fairly firm, yellow appearance, very closely resembling in consistency and colour good, firm, freshly churned grass butter, or have a greenish-yellow colour very like wax." If grey, its vitality is low; if reddish brown, dark or dried and shrivelled, it is dead. Haberland has proposed a test for vitality which consists in placing a drop of strong sulphuric acid on the germ and observing its action. Healthy, vigorous germs quickly assume a fair rosy tint, feeble germs become brown, dead ones black.

Vegetative Energy and Vegetative Capacity.—The former of these expressions is used to denote the percentage of grains in a sample of barley which when placed under favourable conditions for germination, vegetate within a definite time; this at the ordinary temperature, is generally taken as three days. By the latter term is expressed the percentage number which are found capable of germinating irrespective of time. In a good sample of barley the vegetative energy should be not less than 90 per cent., and the capacity not below 95 per cent. The more closely these two characters correspond the better, since any considerable variation indicates that such a sample would grow irregularly on the floor.

Age.—The vitality of barley seems to improve for several months after it has been harvested, and then slowly to decline; so much is this the case, that it is unsafe to malt barleys which are more than two seasons old. Lots which have been stored in a damp condition suffer most in this respect.

Condition of the Endosperm.—When a grain of barley is bitten or perfectly cut in two by a mechanical cutter, the so-called "Korn-Prüfer," the contents of the endosperms, in samples which are capable of being converted into good malt, are of a white and friable or mealy nature. Excessive hardness or any variation in colour from white are indications that such samples will require special care in malting. Grain which is very resistant to the bite, and the fracture of which has a vitreous appearance, can never be converted into good malt. As nearly all malts contain a certain proportion of corns of these two latter classes, it is well to ascertain the respective numbers of each variety in a sample and value it accordingly. This can readily be done with a "Korn-Prüfer," which cuts through 50 corns with one stroke of the knife. It has been found that light will pass through glassy but not through

mealy barley-corns, and upon this fact there has been founded a method of discriminating between the two. The distinction is so sharp that the mealy portions can be detected in corns which are partially glassy. Several appliances have been constructed for carrying out this test, one of the simplest of which is that of Vogel (Fig. 81). It consists of a quadrangular

FIG. 81.—Vogel's Apparatus for Testing Barley for Transparency.

box made of sheet metal, in the interior of which there is a petroleum lamp. The roof of the apparatus is provided with a number of slits, into each of which a barley-corn is placed. The quality of their contents is adjudged from their relative transparency. A somewhat different form of this apparatus is now made under the name of "diaphanoscope." In an examination of barley, by either the Korn-Prüfer or the transparency test, it is usual to operate on 500 corns. These are divided into three classes—floury, vitreous, and semi-vitreous. The numbers thus obtained divided by five give the respective percentages of the different classes.

Coldness.—A sample of barley when handled should not feel cold to the hand, for this is a sign that it is either damp, or immature, or has not been sufficiently sweated in stack. Damp barley stores badly; it is liable to become mouldy, and to rapidly lose its vitality. In addition to this, in buying barley which contains an excess of water by weight, this excess of water represents so much loss and the price should be regulated accordingly. Dampness in the sample which is good in other respects may be removed by sweating—that is, drying it for several hours on the kiln at a low temperature. All barley should be sweated previous to malting, especially at the commencement of the season; but it must be kept in store after this for about a month before it is steeped.

Maturity.—Barley should neither be under- or over-ripe, for in either of these conditions it germinates badly. Immature corn feels cold to the hand, has a sickly greenish or greenish yellow colour, and, when dry, a starved, wrinkled appearance. Over-ripeness in barley is distinguished with difficulty; the skin of such corn has often a dead white appearance, but little difference can be detected in the condition of the endosperm. The degree of ease with which a number of corns slip through the fingers affords some slight insight into the maturity of a sample; mature and dry grains slip through more readily than unripe or damp.

Correlation of "Mealiness" and Density in Barley.¹—Barley grains were placed in a mechanical cutter in such a manner that the distal ends were left in the trays. The completely "mealy" and completely "steely" distal halves were picked out, about 500 of each being collected; their specific gravity was then determined in toluene. The following are the results:—

¹ H. T. Brown, T. B. Case, A. McMullen, J. H. Millar, and F. Escombe (*Trans. Guinness Research Lab.*, 1903, 1, 99).

Barley.	Specific gravity (water = 1).		Percentage of water.	
	Mealy grains.	Steely grains.	Mealy grains.	Steely grains.
I. Archer's (a) . . .	1.305	1.341	15.3	15.5
" (b) . . .	1.303	1.349	15.3	15.5
II. Goldthorpe . . .	1.305	1.346	15.0	15.3

It is pointed out that such differences as are shown in the above table cannot be attributed to varying percentages of moisture in the grain, since the moisture is practically identical in the two cases. Nor can they be explained by differences in the air-spaces between the paleæ and the kernels. Brown and his colleagues accept Johannsen's observation that "mealy" appearance is dependent on interstitial air-spaces.

Microscopical Differences between "Mealy" and "Steely" Grains of Barley.¹—A thin section of a thoroughly vitreous or "steely" barley corn, when examined under the microscope, no longer exhibits a translucent appearance, but shows all the "mealy" properties as completely as if the section had been cut from a "mealy" grain. The conclusion drawn by Brown and his colleagues from their microscopical observations of sections of "steely" and "mealy" corns, is that the "mealy" appearance of the endosperm of barley is directly due to the formation of interspaces around the cell-contents, and within the cell walls themselves. These interspaces are, no doubt, under ordinary conditions filled with air, but they can also be produced under conditions which point to their being at times vacuous, or partly so. They appear to have their origin in stresses and strains induced in the endosperm by its gradual loss of water, a break of continuity taking place with the production of this system of interspaces when the cohesive power of the heterogeneous cell-contents falls below a certain point. Probably the most important factor in producing the stresses and strains is the shrinkage of the starch granules as they fall in water-content from about 40 to 15 per cent. Actual discontinuity, however, in the cell-contents can only take place when the tensile strength of the protoplasmic matrix in which the starch granules are embedded has been surpassed, and it might be anticipated from this that cells which contain the larger amount of protein material would probably best resist the internal stresses and strains, a deduction in close agreement with the fact that "steely" grains are, as a rule, decidedly richer in protein than "mealy" grains (compare p. 435).

Artificial Production of Mealiness in "Steely" or partly "Steely" Grains.²—The coefficient of mealiness of a barley is determined as follows:—Five hundred corns are cut transversely and divided into three classes—"mealy," "half mealy," and "steely"; the percentage of each class in the

¹ H. T. Brown, T. B. Case, A. McMullen, J. H. Millar, and F. Escombe (*Trans. Guinness Research Lab.*, 1903, 1, 100).

² *Trans. Guinness Research Lab.*, 1903, 1, 102.

sample is then noted. If the number 100 be taken to represent complete "mealiness," 1 complete "steeliness," and 50 the intermediate class, the sum of the percentages of each multiplied by its special value and divided by 100 will be the coefficient of "mealiness." It has been observed by Johannsen and by Munro and Beaven that steely grains of barley can often be rendered "mealy" by being steeped in water and subsequently dried at 45° C. (113° F.). Brown and his colleagues found that a very steely Scotch Chevallier, when treated in this way, had its coefficient of mealiness raised from 29.7 to 87.1, the specific gravity of the sample falling at the same time from 1.417 to 1.289. In another series of experiments of a similar nature on nineteen samples of barley from various sources, the average "coefficient of mealiness" before treatment was 52, and rose to 76 after treatment. The visible signs of "mealiness" do not arise simultaneously throughout the endosperm. The first indications of the change are generally seen in the amyloferous cells at the inner angle of the furrow close to the funiculus, and from this point they extend centrifugally and symmetrically until the peripheral layer is reached. Sometimes the mealy appearance invades one lobe of the endosperm more rapidly than the other, and change may apparently start from certain points near the periphery as well as at the furrow, and occasionally the change originates simultaneously at many detached centres in the body of the endosperm, thus having the effect of producing a fine mottling or graining on the surface of the section. These are, however, exceptional cases, centrifugal progression from the neighbourhood of the funiculus being by far the most common mode. It is shown to be necessary in order to obtain the maximum effect in the production of mealiness to dry slowly after steeping.

The Air Content of "Mealy" Grains.¹—E. S. Beaven has proposed to determine the air spaces in the grain from its specific gravity. If the weight of an intact grain be expressed by W and the apparent density of the grain, determined by displacement in toluene or some other convenient liquid, by D , the total volume of the grain will be represented by W/D . This volume, which may be denoted by V , is made up of: (1) that of the dry air-free substance of the grain; (2) that of the moisture; and (3) the volume of the interspaces, which may be filled with air at the normal pressure and may be in part vacuous. Taking w as the weight of the dry substance in W , d the density of this dry substance, and m the weight of moisture in W , the total volume of the interspaces in the grain will be represented by

$$\frac{W}{D} - \left(\frac{w}{d} + m \right),$$

and this can be expressed as a percentage on V .

It is pointed out by Brown and his colleagues that it is usual to take the density of air-free barley substance in water and to assume that the specific volume of the water of imbibition is the same as water in the liquid state, whereas the marked exothermal effects which occur when dry starch or dry

¹ *Trans. Guinness Research Lab.*, 1903, 1, 109.

grain is mixed with water indicate that there must be some contraction of volume going on under these circumstances,¹ and this will tend to reduce the value of *m* and increase the apparent volume of the interspaces in the grain. This contraction phenomenon will, moreover, affect the estimate of the density of air-free barley substance itself, if this determination is made in water instead of in some inactive liquid. These errors tend to make *m* and *d* much too large, and although, since one is a *plus* and the other a *minus* error, they may, under certain conditions, compensate each other, this is merely an accident depending on the magnitude of *m*, or, in other words, on the moisture of the grain under experiment. Data on the density of starch or air-free barley substance are given below.

Experiments on the Density of Potato Starch Containing Varying Amounts of Moisture.²—The specific gravity of dry potato starch taken in toluene, benzene, or ether is 1.5095; taken in water it is 1.6418. Brown and Morris have shown that a considerable condensation occurs accompanied by the evolution of heat when starch is mixed with water.

Density of Air-Free Substance of Barley Containing Variable Amounts of Moisture.³—Barley was ground as finely as possibly in a Maercker Mill, and its density determined in toluene or xylene, dried over sodium. The following results were obtained with (1) a very mellow Irish Chevallier barley having a coefficient of mealiness of 91, and a total nitrogen content of 1.37 per cent., and with (2) a very coarse Northampton barley with a coefficient of mealiness of 47, and a nitrogen content of 1.84 per cent.

Number.	In dry toluene or xylene.		In water.	
	Density.	Specific volume.	Density.	Specific volume.
(1)	1.478	0.676	1.588	0.629
(2)	1.473	0.678	1.584	0.631

From a consideration of the above results it is evident that the contraction of volume which a dry barley undergoes when it imbibes water must be taken into account in any indirect method of determining the air-space in barley. In the provisional formulæ given on p. 438, a coefficient *c* representing the *specific volume* of the water at the particular degree of moisture content of the grain, must be introduced. The corrected expression representing the air-spaces will then be

$$\frac{W}{D} - \left(\frac{w}{d} + mc \right).$$

The value of *c* for barley is practically the same as that for starch, which is given for different moisture contents in the last column of the following

¹ Brown and Morris, *Journ. Fed. Inst. Brewing*, 1899, 5, 338.

² *Trans. Guinness Research Lab.*, 1903, 1, 111.

³ *Ibid.* 114.

table. Since, however, the density of the air-free substance of all barleys is practically the same, a table of the specific volumes (*i.e.*, the reciprocals of the densities) of air-free barley substance containing progressive percentages of moisture has been drawn up.

Specific Volumes of Air-free Barley Substance containing varying Amounts of Moisture.

Moisture per cent. by weight.	Specific volume <i>v</i> .	Difference for 0.1 per cent. of moisture.
0	0.6770	—
1.0	0.6780	0.00010
2.0	0.6790	0.00010
3.0	0.6800	0.00010
4.0	0.6810	0.00010
5.0	0.6820	0.00010
6.0	0.6831	0.00010
7.0	0.6842	0.00010
8.0	0.6852	0.00010
9.0	0.6860	0.00008
10.0	0.6870	0.00010
11.0	0.6880	0.00010
12.0	0.6893	0.00013
13.0	0.6913	0.00020
14.0	0.6966	0.00053
15.0	0.6991	0.00025
16.0	0.7016	0.00025

Application of the foregoing Considerations to the Determination of the Interspaces of Barley by Indirect Means.¹—Representing the specific volume of the intact barley as determined by displacement in toluene or xylene by *V*, and the specific volume of the air-free barley substance of equal moisture content by *v* (see above table), then $\frac{V-v}{v}$ represents the interspaces in unit volume of the barley, and $\frac{100(V-v)}{V}$ the percentage of the volume of the grain occupied by these interspaces. The following results were obtained with a sample of Archer's Chevallier barley:—

	I. "Mealy" distal halves.	II. "Steely" distal halves.
Specific gravity.	1.305	1.341
" volume (<i>V</i>)	0.7662	0.7457
Moisture (per cent.).	15.3	15.5
Specific volume <i>v</i> of air-free barley substance containing 15.3 and 15.5 per cent. of moisture respectively	0.6998	0.7003
Interspaces per cent. by volume.	8.66	6.08

The difference between these two values for the interspaces, namely 2.58 per cent., will approximate the amount of interspaces in the endosperms of

¹ *Trans. Guinness Research Lab.*, 1903, 1, 119.

the mealy grains. Taking the total volume of interspaces in the mealy halves as 100, it is evident that $\frac{25.8 \times 100}{8.66} = 29.8$ per cent. of the interspaces are due to the endosperms, and 70.2 to the envelopes.

Applying the same method to a sample of Goldthorpe barley, the following results were obtained:—

Interspaces in "mealy" halves	8.69 per cent.
" "steely" "	5.89 "

This indicates 32.3 per cent. of the total air-spaces in the mealy halves of this particular barley as being due to the endosperms, and 67.8 per cent. to the envelopes.

It is fair, therefore, to assume with the reservations already made that in perfectly "mealy" grains the distal halves (which naturally do not include any part of the embryo) have about 30 per cent. of their interspaces in the endospermous portions of the seeds.

A Cytological Test for Physiological Maturation.¹—Brown and Escombe² found that as the starch-containing cells of the barley endosperm approach maturity, the nuclei undergo considerable deformation, and are ultimately quite disintegrated by the time the cell is apparently fully matured. From this point onwards these starch-bearing cells appear to be incapable of initiating any “vital” changes within themselves, all subsequent changes in their cell contents at the various stages of germination being induced either by the cells of the “aleurone layer,” which retain their nuclei and vital activity, or by the growing embryo itself. A large number of observations have now been made on the “senescence” and disintegration of these nuclei with the object of ascertaining if the suggestion of Brown and Escombe that this might be made a test of maturation really holds good. Brown and Escombe’s³ method of dissolving out the starch granules with saliva was used, and also intact sections have been mounted in Canada balsam, in which medium, on account of the refractive index, the starch granules are rendered transparent. This latter plan has the advantage that the nuclei may be seen *in situ* without any risk of displacement. The best differential stain was found to be hæmatoxylin (Delafield’s), eosin being used as the contrasting stain for the protoplasmic matrix. Methyl green (acetic) and iodine green with borax and alum, stain well, but the disintegrated nuclei are apt to become decolorised during their subsequent treatment. Since, under the last conditions, the perfect nuclei of the “aleurone” cells retain this stain, it is probable that true chromatin is absent from the disintegrated nucleus of the endosperm. The hæmatoxylin treatment is only fast, because it is precipitated during the subsequent treatment.

Four different varieties of barley, Chevallier, Archer's, Goldthorpe and Standwell, were employed in the first series of experiments. Specimens of

¹ *Trans. Guinness Research Lab.*, 1903, 1, 121.

² *Proc. Royal Soc.*, 1898, 3; *see Journ. Fed. Inst. Brewing*, 1898, 4, 264.

3 *Loc. cit.*

these were taken from the fields on July 20 (1901), July 24, July 29, August 2, August 6, and August 10, the last sample being taken a day or two before harvesting. The samples were prepared in spirit, and represent a range from very immature grain to that which was to all appearances perfectly ripe. Indications of a change in appearance of some of the nuclei of the endosperm cells had already commenced on July 20, but these were only noticeable in the starch-containing cells near the periphery immediately underlying the outermost amyliiferous layer, the so-called "*Kleberschicht*" of Grüss. At the outset the nuclei merely showed signs of a general deformation, evidently produced by the squeezing action of the starch granules as they increased in size and became more closely packed together. This deformation, however, was followed by complete disintegration of the nucleus, and, meanwhile, the action rapidly advanced centripetally, so that within 7 or 8 days nearly the whole of the endosperm had been involved. The last portion of the starch-containing cells to undergo this change were those of the "*Kleberschicht*" itself, the layer immediately underlying the aleurone layer, and containing smaller and less-closely packed starch granules than the more deeply-seated cells. In the later stages of ripening, however, the cell nuclei of the "*Kleberschicht*" were also found to be involved, the very last cells of all to undergo this nuclear "senescence" being those of the "*Kleberschicht*" in the ventral folds on either side of the furrow.

The course of this action is shown by diagrams, and it is pointed out that there is a close correspondence between the microscopical observations and those based on the ordinary criteria of maturation such as are afforded by the general appearance, nitrogen content, and relative "mealiness" or "steeliness" of grains from the same sample of barley, or from barleys grown under identical conditions. When the comparison is made in this way, there is found to exist a distinct correlation between the degree of "nuclear senescence" and the "mellowness" of the endosperm contents which is generally regarded as a criterion of maturation. The nuclear test as a criterion of ripeness is tedious and difficult, and on this account it is not likely to come into general use, but it has the advantage of distinguishing a barley which is physiologically mature from one to which the process of "artificial maturation" (see p. 437) has been applied, which process does not induce any further change in these nuclei, as they still remain witnesses of incomplete ripening.

"Coarseness" of Barley, and its Correlation with other Qualities.¹—Brown and his colleagues find that if a given sample of barley be carefully graded according to "coarseness," the coarser fractions exhibit a less degree of mellowness, and a higher nitrogen content, whilst the average weight and volume of the corns tend to increase with the degree of "coarseness." It appears that in Chevallier barleys of different origin, and widely different as regards "fineness" and "coarseness," there is practically no difference of thickness in their respective "aleurone-layers," or in the thickness of the closely adherent testa or pericarp. As regards the outer integument, the coarse samples

¹ *Trans. Guinness Research Lab.*, 1903, 1, 127.

certainly do show a slightly increased thickness of the paleæ, but it is not very strongly marked. If, however, the combined thickness of the integuments, including all air spaces, are taken into account, very notable differences are exhibited between the "fine" and "coarse" grains. It is evident that these are due not to marked differences of thickness of the integuments themselves, but to the larger air spaces lying between the palea of the "coarser" grains and the underlying pericarp. In the coarser grains the integument is thrown into larger and coarser folds, which enclose on their under side more voluminous air spaces, and the areas of contact of the paleæ with the underlying pericarp are less than they are in the finer grains. This results in a comparative want of optical contact of the paleæ with the pericarp, a fact, no doubt, largely responsible for the peculiar and characteristic appearance of the outer skin of coarse barleys. Although the appearance of "coarse" barleys is not due to appreciable difference in the thickness of the outer envelope, so that the term "thick skinned," as applied to such cases, is certainly not correct, the investigations of the authors point to the fact that ordinary buyer's methods of judging barley by external appearance can be well justified, since there is a close connection between these appearances and the intrinsic qualities of the underlying kernel.

Mouldiness.—It is hardly possible to obtain barley absolutely free from mould, but it should be as nearly so as possible. The more it is contaminated in this way, the more liable it is to become excessively mouldy during the malting process.

Damaged Corn.—Barley may be so damaged as to have its vitality destroyed in several ways. When in store it may be attacked by vermin and insects which partially or totally destroy a number of corns. In careless threshing or dressing many corns may be damaged by being cut in pieces, having their skins partially stripped off, or portions of the corn removed along with the awn. All corns damaged in this way, as well as dead corns, invariably become mouldy during the malting process, and form centres of contagion from which mouldiness spreads to the rest of the barley on the floor. E. R. Moritz¹ has drawn attention to the ill-effects of close dressing, and more recently the matter has been brought before the Highland and Agricultural Society, chiefly through Montagu Baird² who with C. H. Babington was instrumental in inducing the Board of Agriculture to take up the matter and to publish a leaflet recommending more careful methods of threshing barley.

Odour.—Only sweet barley, that is, barley possessing no objectionable odour, will yield first-class malt. Corn having a mouldy, fusty, or other objectionable smell is a highly undesirable article. Barley which is free from the defects previously enumerated, and which possesses the good qualities indicated, is fitted for the production of first-class malt; but in addition to these qualities, there are others which, if not absolutely indispensable, are highly desirable.

¹ *Journ. Fed. Inst. Brewing*, 1895, 1, 228.

² *Ibid.* 1905, 11, 431.

Size.—This may be considered from two points of view—that of absolute size of the individual grains, and the relative size of the grains to one another. Obviously, if each corn were a perfect sphere, such a form would allow of the largest proportion of contents to husk; consequently, the fatter and plumper the grains are, or the nearer they approach this figure in form, the larger the proportion of contents to husk. As it is the contents alone which yield extract, the plumper the grains, other conditions being equal, the more extract will a given weight yield. The more uniform the individual grains are in size, the more regularly they grow; hence the value of grading where considerable difference exists in the size of the individual corns.

Weight.—Barleys vary much in weight per bushel, heavy samples weighing as much as 56 lb. per bushel, light ones not more than 50 lb. Much importance was formerly attached to the weight per bushel; but, providing the more essential characters are present, viz., vitality, friability, &c., light barleys will yield equally good malt. This is especially the case with the thin foreign barleys, the malt from which yields sound worts, especially distinguished by rapid clarification of the resulting beers. The weight per bushel of barley is generally ascertained by a form of instrument called a “chondrometer,” which consists of a small balance provided with a small measure, a funnel for filling, and a strike. The measure is filled by means of the funnel with a fair average sample of the bulk, from which the coarser impurities, such as stones, have been removed. The surface is levelled with the strike, and the weight ascertained. The weights bear the same proportion to the measure as pounds do to the bushel, therefore the weight in pounds per bushel is read off directly.

Uniformity.—As different-sized corns, when steeped, are not evenly wetted, they consequently germinate irregularly; in a first-rate sample of barley the individual corns should be pretty much of the same size. This is a difficulty which can be readily got over by grading the barley with one of the numerous machines constructed for this purpose; and as no sample of barley is perfectly uniform in size, it is advisable to grade all barley before proceeding to work it.

Colour.—Barley should be of a light straw-yellow colour; a greenish tint indicates unripeness, too pale a colour over-ripeness. The tips should have the same colour as the skin; dark or black tips are a sign of heating in the stack, or that the sample has been harvested during wet weather, or that it is old. Such corns grow irregularly at best, and have often entirely lost their vitality. Sometimes the barley is dried on the kiln, and sulphured to improve its colour and odour. This treatment with sulphur is very objectionable; if the light colour of a sample leads to the suspicion that it has been bleached in this way, it should be submitted to a chemical examination, which will reveal the presence of traces of free sulphur and sulphuric acid on it.

Skin.—Good barley should have a thin skin, smooth, and slightly wrinkled. Thick, harsh, rough, crinkled skins indicate that the barley has been grown on cold heavy land.

MALTING.

Malt, more especially that made from barley, is not only an essential ingredient of beer, but it should form the chief portion of the grist; it was correctly described by the late Hofrath Lintner as "*the soul of beer.*" Although adjuncts, such as sugars, and various forms of starch, are exceedingly useful, and, for the production of the modern light ales, practically indispensable, it is impossible to brew satisfactory beer unless the grist used consists in the main of malt. If adjuncts are employed beyond a certain percentage, the beer loses its character. It has been said that "beer is made on the malt kiln" and there is much truth in the adage. At all events whilst it is comparatively easy to brew passable beer from good malt, this cannot be done even by the very best brewer from bad malt. From this fact alone it will be seen how necessary it is that the brewer should possess a knowledge of malting. Indeed one of the qualifications of a brewer should be that he has received an efficient training as a maltster.

The malt-house consists of a main building two or more storeys high, and of a drying kiln, together with stores for barley and malt. Malt-houses are built in many shapes and sizes, the preferable form being that in which the plan of the house is a long parallelogram, with the cistern placed at one of its short ends, and the kiln at the other. The cistern, which may be of brick lined with cement, of wood, of lead, or of cast-iron, is provided with a false bottom perforated with holes; this, acting as a sieve, allows the water to run away whilst retaining the grain. The cistern should have a capacity of about twelve cubic feet for each quarter of barley to be steeped. In the construction of the floors many materials have been used; the principal requisite being that the floor has a smooth, even surface, which can be readily cleansed and which will not absorb moisture too readily. Probably well-faced Portland cement makes one of the best floors. One hundred and eighty to two hundred square feet of floor space should be provided for every quarter of barley to be steeped. The uppermost storey is generally used as a store-room for the barley, from which it is shot down into the cistern at each steeping. After being steeped it is cast out, heaped up into couch and then spread on the floors to germinate. In more modern houses the uppermost storey, in addition to forming the barley store, also contains the machines for cleaning and grading the barley, and the steeping cistern or cisterns as well. These are in this case made of iron, the lower portion being constructed in the form of an inverted cone, which has outlet valves at its apex whereby the water and grain may be let off independently. This form of cistern secures considerably better drainage, and as the barley when steeped is simply discharged to the place where it is required, all damage from shovelling being avoided. An abundant supply of water should be provided for each cistern, and also a drain to take away the steep-water. Every cistern should be provided with some arrangement by

means of which the water can be warmed to a slight degree in very cold weather.

General Principles of the Malting Process.—Briefly stated malting consists in the partial germination of raw grain by causing it to imbibe a certain amount of water and by keeping it under proper conditions on a floor or in special drums. After germination has proceeded to the desired extent, the rootlets which have developed are allowed to wither; this happens as a consequence of the loss of moisture by assimilation and by evaporation. The grain is then transferred to the kiln, where it is first dried at a temperature ranging from 80° F. on the first day to say 150° F. on the third day, after which the temperature is raised and the malt cured. The temperature on the third day as well as that at which the curing is conducted depends on the class of malt to be produced (see p. 463). During germination the secretion of diastase,¹ and other enzymes takes place, and these acting on various parts of the endosperm furnish the necessary germ food for the growth and respiration which are taking place. At the withering stage the rootlets die off and little or no further growth of the acrospire takes place, for the grain is in a moribund condition; the diastase and other enzymes, however, which at that time are distributed throughout the whole of the endosperm act upon certain of its contents and produce the condition known as mellowness. This mellowing is completed at the initial stages of the kilning process, whilst at the intermediate and final stages of kilning the restriction of diastase and the production of colour and of specific flavours are brought about. The various stages of the conversion of raw grain into malt will be considered in greater detail *seriatim*.

Screening.—Barley, before being steeped, should be screened by means of one of the numerous machines which are on the market for this purpose. These not only remove the dust and dirt, but also separate the small and broken corns, and grade the barley into different sizes. This last is a matter of considerable importance when a quantity of barley irregular in size has to be dealt with, for unless the individual corns are pretty equal in size, some absorb more water than others, and, as a consequence, irregularity of growth ensues.

Steeping.—The barley, after being screened, &c., is immersed in water in the cistern, in order that it may absorb an amount of that liquid sufficient to arouse its dormant vitality and set in action the train of physiological processes which are collectively included under the term "germination." The amount of water which different samples of barley will absorb in a given time varies much with their size, condition of skin, &c.; consequently, a certain amount of judgment, only to be arrived at by experience, has to be exercised by the maltster in this operation. About fifty hours may be taken as the average length of time of steeping for English barleys, but, as has just been stated, this will vary much according to the nature of the barley and

¹ The diastase of raw barley appears to differ essentially from the diastase of secretion of malt (see Brown and Morris; also Baker, p. 124).

the temperature of the steep-water. The light Danubian and Odessa barleys require about sixty hours' steep, and the Smyrnas about seventy-two hours. The proper quantity of water which should be absorbed is about 50 per cent. In steeping, the barley increases considerably in bulk, one quarter expanding to a volume of from 1.18 to 1.20 quarters. In conducting the operation of steeping, it is best to place the proper quantity of water into the cistern, and then to run in the barley in a slow stream, stirring vigorously all the time. In this way the contact of every grain with the water is assured, and any dust or refuse which floats on the surface of the water can be removed.

Change of Steep-Water.—As water dissolves a certain amount of organic and mineral matters from the barley, and this affords a supply of food for the numerous bacterial organisms which are always adherent to the barley, the steep-water sooner or later becomes putrid, and requires changing. R. E. Free says:¹ "Without counting the water in which the grain is primarily steeped, the grain should certainly be drained at least three or four times while it remains in the cistern." Barley should never be over-steeped or sodden, as, in such a case, the vitality of some grains is considerably weakened and that of others completely destroyed. Deficiency of water can be afterwards remedied by sprinkling on the floor, but it is almost impossible to remedy the ill effects of over-steeping. Some maltsters find it advantageous for preventing mould to use bisulphite of lime in the steep-water, at the rate of about one gallon of the commercial solution to every thirty quarters of barley.

Signs of Proper Steeping.—When a corn of properly steeped barley is taken by its ends between the finger and thumb, the exertion of a certain degree of pressure in squeezing it together should cause the skin to leave the grain. If it yields to pressure too easily, it is over-steeped; if too resistant, it is insufficiently steeped. When a grain is cut through with a sharp knife, the whole contents should appear evenly wetted, but should not be soft and milky. It should be possible to pass an ordinary sewing needle easily through the grain.

Temperature of Steep-Water.—This is a point of considerable importance, and one which is very often overlooked. The subsequent growth of barley is much prejudiced by being submitted to too low a temperature in the cistern. The temperature should be between 50° and 55° F., and should never fall below the first of these figures. On the other hand, a temperature above 56°F. favours bacterial action, as evinced by the steep-liquor rapidly becoming sour or putrid.

Quality of Steep-Water.—It has been a much debated question as to whether steep-water should be soft or hard; the former extracts more potassium phosphate, the latter more organic substance. An excessive quantity of sodium chloride (above 10 grains per gallon) is said by Maercker to retard germination and to cause an imperfect development of the rootlets, the malt when finished being deficient in extract and containing a high percentage of

¹ *Trans. Inst. Brewing*, 1891, 88.

nitrogen; excessive amounts of calcium chloride or magnesium chloride appear to act in the same manner. Ferruginous waters are also unsuitable. The presence of nitrates in moderate quantities appears to be favourable to germination. Prior¹ considers that any fairly pure water which is free from the above-mentioned salts may be used for steeping—preference being given to soft water, or to a moderately hard water in which the hardness is due to calcium sulphate. Many maltsters use a certain proportion of lime water in the steep. One of the authors (A. R. Ling) has found from practical experience that this promotes regularity of germination. Working with a moderately soft water, he has for some years mixed one-third of lime water with it for the first change, the other changes being the plain water. This appears to be the best means of applying lime in the steep. On no account should solid lime be added to the cistern.

The Changes which Take Place during Steeping.—During this process the grain absorbs a large amount of water; its quantity has been given by Ford as an average of 47 per cent. on the original weight of the grain—that is, 100 lb. of barley, after being steeped, and with the surface moisture removed, would weigh 147 lb. Thausing gives a somewhat higher figure than this—from 50 to 55 per cent. with a barley containing originally 12 per cent. of moisture; but the amount varies with each sample of barley, and its condition as to dryness. Stopes² states that 20 per cent. of imbibed water is sufficient to start germination, and that 30 per cent. is sufficient to carry some barleys through the whole process of malting, though others require 40 per cent., and some even more than 50 per cent. The grain also, as already stated, considerably increases in bulk after being steeped. According to Holtzner, the water finds its way into the barley partly through the husk, but chiefly through the apertures at each end of the grain, between the husk and the grain; it thence passes by diffusion from the periphery of the grain to its centre. A certain amount of various substances, organic and inorganic, such as cane sugar, gum, diastase, colouring-matters, &c., as well as a portion of the mineral constituents are dissolved out of the barley by the steep-water. The total amount of the organic matters removed in this way has been estimated by various observers at from 0.6 to 1.5 per cent., and about one-tenth of the mineral substance is similarly lost. The quantity so dissolved will vary according to the temperature of steeping, the condition of the barley, and the quality of the water employed. The amount of acid originally present in the barley is increased by the formation of fixed organic acids. But trifling quantities of phosphoric acid are extracted in this way; so small are they that the percentage of this acid may, owing to the comparatively larger amount of the other substances dissolved out, be greater in the steeped barley than it was in the original corn. About one-half of the soluble nitrogenous constituents of the barley pass over into the steep-water. Many of the spores of bacterial organisms and of the mould fungi, &c., which are always present in abundance on the husk of the grain, are washed away. Steeping is not a mere

¹ *Chem. u. Physiol. Malzes u. d. Bieres.*, p. 98.

² *Malt and Malting*, p. 334.

mechanical process, consisting in the imbibition of a certain amount of water. Physiological changes are undoubtedly initiated while the grain is in the cistern. Some evidence that constitutional changes occur during steeping is furnished by the observations of Baker and Dick,¹ who show, by co-ordinating the amount of water taken up by the grain and the time in the graphic method, that breaks in the curve are exhibited. Thus a sample of Californian Chevallier barley contained 26.4 per cent. of water after steeping for 19 hours, but this was reduced to 24.9 per cent. at the end of 20 hours. After this an increase in the water again took place. This is typical of several examples given by the authors.

Àération of the Steep.—According to Stopes² a practice is adopted in Bohemia of steeping for 20–24 hours in a cistern and then couching at a depth of about 10–12 inches. The corn remains in this couch for another 24 hours when it is returned to the steeping cistern and again submerged. This process, which practically amounts to aëration of the steep, is approved by Dr. Graham.³ Later Stopes⁴ states that change of water during steeping is to be recommended whether it becomes fœtid or not, since the chief advantage is derived from the access of air to the grain throughout the steep. Moritz and Morris⁵ recommend that the grain should be well aërated in the steep. As a result of much research, principally in Germany, the beneficial effect of aëration during steeping has been established. We are indebted to W. Windisch and to Bleisch and Will for some valuable work on this subject. They have shown that when aëration is practised sprouting or chitting of the grain commences much earlier, and that there is a gain of two days on the flooring period, enabling germination to be carried out at a much lower temperature. In addition to the saving of time evidence has been brought forward by German maltsters that malting losses are reduced when the grain is aërated in the cistern. Baker and Dick⁶ have published experiments showing the advantages of aëration of the steep, and their opinion that the subject is one which deserves investigation at the hands of English maltsters must be endorsed. They state that inasmuch as sprouting usually occurs two days earlier when aëration is practised, germination on the floor can be conducted more slowly, and consequently at a lower temperature, with the result that the malt is not forced. G. Cecil Jones has expressed himself in doubt as to whether malt made under the stimulus of an aërated steep might be described by some brewers' chemists in this country as having the character of a forced malt; and Lawrence Briant has observed that in more than one instance he had found that, whilst with aëration there was a more vigorous and rapid growth in the earlier stages, there was a peculiar falling off in the later stages necessitating the use of a considerable amount of sprinkling water. The experience of J. Stenhouse is entirely

¹ *Jour. Inst. Brewing*, 1905, **11**, 380.

² *Malt and Malting*, 1885, p. 312.

³ *Cantor Lectures, Society of Arts*, 1874.

⁴ *Trans. Lab. Club*, 1888, **2**, 52.

⁵ *A Text Book of the Science of Brewing*, 1891, p. 81.

⁶ *Loc. cit.*

favourable to the aëration process, the malt being well modified and not forced.

The fact appears to have escaped notice that inasmuch as sprouting commences earlier when the grain has been aërated in the cistern, the piece will be in a condition for sprinkling before it is in the ordinary process. In fact on account of the more rapid assimilation of water, sprinkling will more likely be required when the grain has been aërated than under ordinary circumstances; and if water is not given when necessary, the rootlet will die off and further growth will be arrested. Too early withering renders it impossible to obtain perfectly modified malt as, besides stunted germination, the secretion of enzymes, respiration and other changes, directly connected with the vital process, will not have proceeded far enough. Malting is frequently carried out on too strict routine lines, and as a case in point, sprinkling is applied when the floor is a certain age whether it be required or not. Rootlet development must have advanced to a certain degree before sprinkling water is applied, otherwise it is of no avail as it is not absorbed. An experienced maltster knows, however, by the appearance of the piece exactly when water is needed.

Among the arrangements for carrying out aëration in the steep must be mentioned that of Doorkaat, which may be applied to a cistern of any form. It consists of a series of vertical ejector pipes, connected with a supply of compressed air. Windisch has remarked that he attributes less importance to the actual aëration of the grain than to the thorough rousing during steeping, and in this connection it is interesting to mention Sleeman's process of steeping in a drum.¹ The inventor claims that by the friction of corn against corn and against the sides of the drum, the grain is thoroughly cleansed whilst aëration is effected most completely. A conical cistern is made by Messrs. R. J. Hanbury and Co. which is furnished with arrangements permitting the thorough aëration of the grain during steeping.

Germination.—In this portion of the operation the two chief objects of the maltster are to secure what is known as the “modification” of the contents of the endosperm, and to generate the enzyme diastase in sufficient amount. This modification of the endosperm constitutes the chief difference between barley and malt; and on its perfect accomplishment depends the amount of substance which will subsequently enter into solution in the mash-tun; in other words, it determines in a great measure what shall be the amount of extract yielded. It also to a considerable extent determines the quality of the dissolved matters. As we have seen before (p. 297), these changes are due to the effects of the physiological processes which take place during germination; an enzyme is secreted by the secretory layer and gradually invades the endosperm. This gradually permeates the substance of the endosperm, partially dissolves up the cellulose walls of the starch-containing cells, and leaves the latter in a condition in which they are more directly accessible to the diastase dissolved out by the mash-liquor. It is upon the action of enzymes that

¹ *Brewers' Journal*, 40, 486, 1904.

the "modification" of the contents of the endosperm mainly depends. But there is much difference in the degree to which its action extends in different barleys; the cellulose of some samples appears to be peculiarly resistant to its influence, and, consequently, such specimens can never be made to yield tender, friable malt. Sometimes, or perhaps it may be said often, through deficient germination, the enzyme does not reach the extreme ends of the corns, in which case a malt is produced, many of the corns of which have hard ends, and all such unmodified masses represent so much raw barley. It is desirable, however, that the fullest action of the diastase should be exerted on the cellulose throughout the whole endosperm. As regards the diastase, though it should be secreted in large quantities, yet its action on the starch must be limited as much as possible, to keep it down, as nearly practicable, to the extreme limit capable of furnishing food to the plant during the period necessary for inducing the requisite changes in the constituents of the barley-corn. If the action be greater than this, an amount of starch is destroyed unnecessarily. The respiration of the embryo must, therefore, be kept within due limits in order to avoid waste, and this is chiefly effected by never permitting the heat of the germinating barley to exceed a temperature of 60° F. All the carbon which is expended in keeping the heat of germinating barley at a temperature beyond this represents so much lost material.

During germination there is an increase in the diastatic activity. This was first pointed out by Kjeldahl. A. Gordon Salamon¹ has published some analytical data on this point, and the following table represents one series of his experiments. The diastatic power is expressed on Lintner's scale in each case and on a common basis of 2 per cent. of moisture.

Day	Diastatic Power.
First	6.5
Second	7.0
Third	10.7
Fourth	49.2
Fifth	78.1
Sixth	74.1
Seventh	—
Eighth	90.4
Ninth	81.3
Tenth	77.4
Eleventh	85.5
Twelfth	108.2
Thirteenth	125.0

It is seen that although there is an increase from start to finish, at certain stages the values show a decrease on succeeding days.

As diastase is able to act upon starch in the cold, it naturally follows that its presence will be attended with the products of its action on the starch of the endosperm. The first sugar produced is maltose, and this according to Brown

¹ *Journ. Fed. Inst. Brewing*, 1902, 8, 2.

and Morris, becomes partially transformed into cane sugar in the embryo by the living cells of the latter. Kjeldahl found that when a barley which originally contained 1.5 per cent. of cane sugar, had arrived at the stage of green malt, it then contained 4.7 per cent. of this sugar. The former observers were able to prove that the largest amount of cane sugar was contained in the germ, whilst comparatively small amounts existed in the endosperm. Thus, of the total amount present in barley, the embryo contained 5.4 per cent., the endosperm but 0.3 per cent.; in the same barley, after ten days' germination, 24.2 per cent. were found in the embryo and 2.2 per cent. in the endosperm. Simultaneously with the formation of cane sugar, a small amount of invert sugar seems to be formed by its hydrolysis, and this accounts for the presence of dextrose and levulose in the finished malt.

In germinating barley we are absolutely obliged to produce a certain quantity of rootlets; these, after the malt has been dried, are removed, and as they are, comparatively speaking, a valueless material, they represent a loss, for the substance from which they are formed is derived from the endosperm. By different methods of germination very different quantities and weights of rootlets may be produced, and it is therefore the maltster's aim to keep root-production to as low a limit as is consistent with securing the proper modification of the barley. The comparative state of wetness or dryness in which the barley is germinated has great influence upon the amount of root-production; if the barley is worked too wet, and especially at too high a temperature as well, rootlet-production proceeds at a high rate. In such a case the growth of the acrospire and the modification of the contents of the endosperm do not keep pace with the root-formation. The rootlet should be curly. A long straight rootlet is spoken of as "wild" and is to be avoided.

The Barley during the Whole Process of Germination Respires.—During the period of germination barley absorbs oxygen and exhales carbon dioxide, and any undue accumulation of this latter gas either stops growth or seriously impedes it. Provision has, therefore, to be made for removing the carbon dioxide and supplying fresh oxygen. This necessary aëration of the barley is secured by the continual turning it undergoes, and by securing the frequent renewal of the air in the malt-house by proper ventilation. At the same time the amount of ventilation must not be too great, for in that case, moisture will be removed from the barley. Again by too frequent turning the rootlets are injured and vitality impaired. Schütt¹ found that, in a period of nine days' germination, about 196 cubic feet or 36 lb. of carbon dioxide were given off by a quarter of barley, which is equivalent to a loss of 22.5 lb. of starch; during the same period 11.5 lb. of water were formed. The amount of the carbon dioxide evolved was found to be slightly greater than that of the oxygen inhaled; this shows that intramolecular respiration takes place to a limited extent. Stoklassa and Czerny have shown that anaërobic or intramolecular respiration, which takes place in the cells of the higher plants and animals is a process of alcoholic fermentation, produced by an enzyme similar

¹ *Woolh. f. Brau.*, 1887, p. 673.

to the zymase of yeast. According to Day,¹ a definite relation exists between the amount of carbon dioxide evolved and the water formed.

As germination proceeds, a considerable portion of the hitherto insoluble proteid bodies is converted into soluble forms; thus Hilger and Van Becke found that, after five days' germination, the soluble proteid matter of a sample of barley was three times as large as that of the original grain, and was nearly four times as large in the finished malt. The amount of acidity of the barley also augments during germination; Belohoubek found an increase of from 0.34 per cent. (reckoned as lactic acid) in the original barley to 0.60 per cent. in the green malt. Prior² determined the respective amounts of the free and fixed organic acids, of the primary phosphates, and of the total phosphoric acid, in two samples of barley during germination. After two days' germination the volatile acids had slightly increased in one sample, whilst in the other they remained the same. The fixed acids had shown a not inconsiderable diminution, and the primary phosphates had increased in an almost exactly proportionate amount. This he considered to be due to the action of the fixed acids on the secondary and tertiary phosphates contained in the grain, which were converted into primary phosphates according to the action expressed in the following equation:

And similarly with the other phosphates of magnesium and calcium. This took place chiefly between the second and fourth days, and caused the rise in total acidity noticeable at this period. An apparently slight diminution in the total amount of phosphoric acid was also observed, and this was thought to be due to an increase in the total weight of the grain, caused by the combination of a small amount of the absorbed oxygen with some of the constituents of the grain. From the second to the fourth day there was a rise in the total acidity, chiefly caused by the formation of primary phosphates; meanwhile the volatile acids remained about the same, but there was a slight decrease in the amount of the fixed acids. A slight increase in the total phosphoric acid also occurred, but this was probably only apparent, being due to some of the combined oxygen being dissociated. From the fourth to the sixth day the total acidity of one malt had augmented, whilst that of the other had slightly diminished. The augmentation was not due to the volatile acids, for these were the same in amount, but to the fixed acids, which appeared to have been released to some extent from the bases which they had before converted into primary phosphates; apparently, then, a reversal of the original action had taken place. From the sixth to the eighth day there was a very slight decrease in the total acidity. The volatile acids were not increased in amount, but the fixed acids had slightly decreased, and

¹ *Journal of the Chemical Society*, 1880, p. 645.

² *Chem. u. Phys. Malz, u. Bier.*, p. 112.

a corresponding increase had again taken place in the primary phosphates. The total amount of phosphoric acid appeared to be slightly higher, probably owing to a diminution in the weight of the other constituents of the grain. The total acidity of the rootlets was twice as great as that of the green malt itself. As a result of a long series of experiments Fernbach¹ contends that the acidity of malt consists exclusively of acid phosphates and that no free acids are present.

Growth of Mould.—Another point of the greatest importance is to prevent the growth of mould while the barley is on the floor. It is impossible to grow barley entirely free from mould, but its growth should be kept down to the lowest possible limit. This is effected by the removal of damaged corns, and by exercising care in steeping, so that the vitality of the corns is neither depressed nor destroyed, by securing an abundant supply of fresh air in the malt-house, and by working the barley without an undue excess of water.

Couching.—We now proceed to the actual operation of germination on the malt-house floor, taking for example what may be considered as the average course of a steeping of barley. The first operation, after the barley leaves the cistern, is known as “couching,” so called because, in the days when the duty was collected on the malt, the barley on leaving the cistern, was placed in the couch-frame, levelled, and there gauged by the Excise officers. The steeped barley is levelled up into a heap of twelve or fourteen inches in depth, either on the couch, where such still exists, or, where it does not, on the floor. Here it is left for a period of about twenty-four hours, during which time it begins to exhibit the first signs of vitality. Now that it is removed from the water in which it was immersed, and is surrounded by air, it is able to breathe, and the evidence of respiration is soon afforded by the rise in temperature of the heap. The barley during this period sweats, and gives off a distinctly pleasant odour not unlike that of apples. It is probable that at this stage alcoholic fermentation occurs and that the alcohol produced interacts with the acids giving rise to ethers. An old writer, J. H. Baverstock, remarks that if at this stage “a portion of the malt be distilled in a steam bath a little spirits will be found in the liquid which comes over.” The barley while on the couch is turned, as a rule, every five or six hours, in order to secure proper aëration, equability of temperature, and an even distribution of moisture. When the heat of the heap has risen to from 60° to 63° F. it is broken down, that is, extended in superficial area and reduced in depth. At this period it will be found that many of the corns show the first visible signs of growth, little white protuberances having made their appearance at the ends of the grains where the germ is situated. When this occurs the barley is said to “chit.”

Flooring.—This portion of the process may be said to commence with the breaking down of the couch, at which time the piece is called a “one-day floor;” the next day it becomes a “two-day floor,” and so on. The parcels of malt are called “pieces,” and each of these is turned, as a rule, twice a day,

¹ *Journ. Fed. Inst. Brewing*, 1896, 2, 128.

but this will vary according to circumstances. The turning is effected by means of large wooden shovels, and is an operation requiring a considerable amount of dexterity, which can only be attained by long practice. When properly effected, the mass of grain is entirely turned over, that portion which formed the surface of the original piece becoming the undermost layer in the fresh piece. In this way a certain uniformity in moistness and temperature is secured. Each succeeding piece is turned on to a portion of the floor farther away from the cistern; consequently, it gradually travels towards its destination, the kiln. The thickness of each succeeding piece is determined by the temperature of the previous one; if the temperature of that was too high, the next is spread out into a thinner layer, or *vice versa*.

Sprinkling.—Probably by about the fifth day the rootlets will begin to lose their turgid appearance, as evidenced by flaccidity, a loss of their bright colour, and an inclination to turn yellow. When this appearance presents itself it is a sign that the piece is becoming deficient in moisture. This deficiency is supplied by sprinkling water over the piece at the rate of from two to six gallons of water per quarter of barley, according to the condition of the piece. The sprinkling is usually effected by means of a can, provided with a long spout perforated with a number of fine holes; or, where water is laid on for the purpose, by means of a hose and fine rose. Probably one or more sprinklings may be found necessary, but this is a point which must be left entirely to the judgment of the maltster. Bisulphite of lime is often added to the sprinkling of water at the rate of two pints per barrel in cold weather, and four pints in warm; it serves to check the growth of mould. As already remarked sprinkling must be carried out at the proper time, but late sprinkling is specially to be avoided not only on account of its causing too advanced germination but because withering does not take place. By thickening up a piece which has been sprinkled at a late stage, sweating sets in and “forced” malt is produced.

Growth.—As before stated, chitting will have commenced in an average steep at the end of twenty-four hours from the removal of the barley from the cistern. On the following day many of the corns will show from one to three distinct rootlets; by the third day nearly the whole of the grains will have from three to four roots; and on the fourth, rootlets to the number of four or five will have developed. It is the object of the maltster to obtain a short, abundant, bushy root consisting of five or six rootlets. Too long rootlets, with too short a growth of acrospire, are a source of waste, for they show that the modification of the endosperm is not keeping pace with the growth of the rootlets.

By the evening of the third day the acrospire will have commenced to move up the back of the grain, and will be distinctly visible; in another three or four days it will be half-way up, at which stage sprinkling is generally commenced; in another two or three days it will have reached a length of two-thirds of the grain. When this point is reached growth continues very slowly with the gradual loss of moisture until incipient withering takes place.

Withering.—The practice of thickening up the piece while the rootlets are still fresh is to be condemned. In this way the malt collects heat and sweats, and although the rootlets may ultimately die off by loss of moisture this is preceded by growth of both acrospire and rootlets to an extent beyond the requisite limits, there being a corresponding loss of carbohydrate matter. As above stated, by working in this way the rootlets do wither and die, yet as R. E. Free very truly remarks the malt, instead of going to kiln with a pleasant sweet smell and in a good condition, emits a peculiar fusty odour similar to damp straw. This is especially the case when, owing to the presence of broken or damaged corns, mould development sets in. Then the maltster has no alternative but to bring the malt to kiln, whether it has been mellowed or not, and very often he is compelled to raise the temperature too rapidly in order to kill the mould.

If the work has been properly conducted, by the time the acrospire is about two-thirds up the back of the corn, that is, as a rule, by the eighth or ninth day, the rootlets will commence to lose their freshness; the exact period depends, however, on the character of the barley and how the early work on the floors has been conducted; it may sometimes be later. Assuming sprinkling to have been necessary and that it has been given at about the fifth or sixth day, after this there should be a gradual loss of moisture which will bring about incipient withering. When this appears the piece may be thickened up to some extent in order to effect mellowing, but on no account should the temperature rise much above 60° F. until the last few hours before the malt is loaded on kiln, and then it is open to doubt whether a temperature of 70° F., as advocated by some maltsters is not too high. Moderate sweating is perhaps permissible in some cases, but with kindly barleys, mellowing can be produced without greatly thickening the piece or raising the floor temperatures. After the appearance of incipient withering the pieces may generally be put into a smaller space, and it is important that for the last twenty-four hours they should remain undisturbed. During this period the piece is not so freely ventilated, and the carbon dioxide exhaled collects and by arresting respiration checks growth. The secretion of enzymes no longer occurs, but these substances permeate the whole endosperm and complete the "modification" bringing about the condition of mellowness. The malt when brought to kiln should, as a rule, contain not more, and preferably less than 40 per cent. of moisture, and it should break down to a floury condition when rubbed between the thumb and finger.

The sugars formed at this stage not being oxidised so rapidly as respiration is checked, accumulate, the more so if temperatures have been allowed to rise to undue limits and hence an excess of these is indicative of forcing as first pointed out by Moritz and Morris (see p. 427).

Pneumatic Malting.—The malt-house of the present day has practically the same general construction which it has had from times immemorial; nor is this remarkable seeing that malting is a biological process. During the last twenty years the spirit of reform has extended itself in a small degree to the malt house, and there have been some attempts at improvement in the construc-

tion of the plant. The introduction of the conical steeping cistern, turning machinery, the various improved forms of apparatus for cleaning and grading

FIG. 82.—Diagrammatic View of a pneumatic malting, showing pneumatic washing and steeping cisterns.

the barley, machinery for the removal of broken corns, &c., are instances. But the first real attempt at radical reform is due to Galland who, in 1874,

designed an apparatus which was intended to obviate the large amount of hand-labour expended in the constant turning of the barley. The steeped barley was placed in a thick layer on a floor, that consisted of perforated iron plates, through which moist, cool air was continually drawn and compelled to traverse the barley. In this way the grain was abundantly supplied with oxygen, and

FIG. 83.—Section of Pneumatic Malting Drum,

at the same time the carbon dioxide was effectually removed. As the air was saturated with moisture, it was expected that it would exercise no drying action on the barley; but, owing to the friction generated, some loss of moisture ensued. This principle has been preserved in all the pneumatic methods of malting which have been since proposed; and obviously, if such a method were found practicable, it would be possible to make malt all the year round, because the air used in the process could be cooled down at any season of the year to the temperature required for malting.

Galland's idea was to do away with the turning altogether, but this was found impracticable, for the mass of germinating barley gradually became matted and felted together, and more and more impervious to the air-current; the rootlets also penetrated and filled up the perforations in the floor, thus offering another obstacle to the air-current. In consequence of this a considerable degree of force was required to drive the air through the mass of grain, considerable friction ensued, and the heat developed in this way tended to remove water from the barley, even though the air employed was saturated with moisture. Some means had therefore to be provided for turning the barley and keeping it in a loose condition, and as the pieces in the apparatus were from thirty to forty inches in thickness, it was impossible to do this by hand. To remedy this defect Saladin invented a mechanical arrangement for turning the barley. It consisted of a number of vertical screws, which, in addition to having a revolving motion on their own axes, travelled from one end of the apparatus to the other. This turning apparatus was put in motion every eight or twelve hours, when it effectually turned the grain and left its surface perfectly level. The apparatus in this improved form has proved fairly successful, and is employed to a limited extent in France and Germany.

Drum Pneumatic Malting.—Galland fully recognised the imperfections of his original method, and, in order to remedy them, proposed to vegetate the grain in a slowly revolving drum, provided with various air-passages, by means of which a constant current of moist air at a suitable temperature could be continuously passed through the germinating grain. Since the barley was being continuously turned, it was kept in a loose condition and not permitted to felt together. This system was further improved by Henning, and in the opinion of some, now bids fair to become ultimately the universal method of malting. The general appearance of a pneumatic malt-house is shown in Fig. 82. One of the drums is shown in section in Fig. 83, together with the arrangements for providing for the circulation of the moistened air. The passage *L*, from which the air is drawn, communicates with the chamber *A*, Fig. 84, where the air is moistened by passage through a spray of water, which may be either cold or warm, according to the temperature to which it is desired to heat the air. The saturated air passes up the tube past the valve *D*, which can be opened or closed at will, to a number of passages, *c*, on the inner surface of the drum, best seen in Fig. 84. (The letters indicate the same portions of the apparatus in both diagrams.) The drum *T* is a cylinder of sheet metal which revolves on its axis, and is supported on the wheels *b b*. The rotation is effected by a worm acting upon a series of cogs which surround the circumference of the drum. The drum is provided with an aperture, which can be closed with a lid, for the introduction of the steeped barley and the removal of the green malt. A tube *m* is fixed along the axis of the drum; this communicates with the valve *D*₁, and with the passage *S*₁. From this last the air is drawn by means of the exhaust fan *Z*. The steeping cistern is shown at *W*₁, one cistern sufficing for two drums; the shoot, as seen in Fig. 84, being

movable. The direction in which the air passes through the germinating barley is shown by the arrows. The slow rotation of the drum (once in forty to forty-five minutes) keeps the grain in a loose condition and readily permeable to air. An arrangement is also provided by which the air-supply can be shut off from the passage *L* and drawn from the outside atmosphere, either directly or after filtration through an air filter.

The chief advantages of the pneumatic system are the command which it

FIG. 84.—Section of Pneumatic Malting Drum.

gives to the maltster in controlling the temperature of the germinating barley, in adjusting the degree of aëration to which it shall be subjected, and in regulating the amount of moisture which the air-supply shall contain. All damage to the corn by turning with a shovel, or by the feet of the workmen, is avoided. Owing to the abundant supply of fresh air the malt is kept absolutely free from mould, and this is no doubt considerably aided by the purification (removal of germs) which the air undergoes in its passage through the coke tower. There is also an enormous saving in space—only one-sixth of that required for the ordinary growing floors. Hand-labour too is reduced to a minimum. Against this must be placed the expenditure for power in

driving the exhaust-fans and drums, together with the pumping of the water for moistening the air.

With regard to the important question as to how the malts made by the pneumatic system compare with those made on the floors, it is very difficult to form a correct judgment. There is still much prejudice among

FIG. 85.—Interior of Messrs. Bass, Ratcliffe and Gretton's Pneumatic Malting.

old-fashioned maltsters in favour of the floor system, and as the pneumatic process has not been tested as widely as it might have been, there is a lack of published data. It cannot be denied that by the pneumatic system the maltster is independent of outside conditions, such as changes of weather and temperature, and that he can work just as successfully in summer as at the cooler time of the year. The process, also, needs a less amount of skill and judgment in carrying it out, owing to its being under complete control. Some points, however, require consideration. Too much air should not be passed over the germinating malt in the drums; the aim should be to produce a curly rootlet by appropriate air-supply and turning. When the process was first introduced, withering with the concomitant mellowing of the grain was not possible. As a consequence of the former the malting loss may have been quite as great or even more than on the floors, and in the absence of proper withering the malts were hard and badly modified. In such malts the soluble albuminoids and carbohydrates other than cane sugar and invert sugar appear to be low, this being particularly noticeable when the drying, as distinct from the curing, has been performed, as was

formerly the practice, in a drum.¹ Despite these facts, however, hard malts of the kind referred to often give good extracts. Recently the process has been much improved, and having been taken up by leading maltsters and brewers, some of the tenderest and best modified malts have been produced by its means. The accompanying illustration, Fig. 85, shows a portion of the interior of Messrs. Bass, Ratcliffe and Gretton's pneumatic malting at Burton.

The Drying Kiln.—This consists of a building having a roof in the shape of a cone, which terminates in a short chimney, usually surmounted by a cowl. The building is divided into two chambers by the drying-floor, which may consist of wire gauze, perforated iron plates, or perforated tiles. In the space beneath the drying-floor is the arrangement for supplying heat, and this, in its simplest form, is merely an iron cage in which the fuel is burnt. In order to equally diffuse the heated air, a large plate, generally made of iron, and covered over with bricks or some other non-conducting material, is placed over the fire. Openings are provided in the walls of the room under the drying-floor; by the opening and closing of these, together with the manipulation of the fire, the temperature of the air passing through the green malt is regulated. Occasionally drying kilns have two floors, one above the other. The green malt is first placed on the uppermost, and, after having attained a certain degree of dryness, is then shot down to the lower floor, where the operation is completed. In this case provision has to be made for the entrance of cold air into the space between the two floors, or some arrangement provided by which the bulk of the heated air coming from the lower floor can escape without actually passing through the grain on the top floor.

In order to increase the volume of air passing through the green malt, especially in the earlier stages of the drying process, it has been proposed by Mr. Free to place a Blackman fan at the apex of the roof. This plan, he says, has been carried out with a considerable degree of success in his own maltings. (Compare, however, p. 463.)

Drying.—The objects of this process are to arrest effectually further growth in the green malt by the withdrawal of water, which has the effect of rendering the malt stable, so that it will keep on storage, and also to confer flavour on it. In no other department of malting is so much care required as in the kilning, for the character of the beer produced by a malt is most essentially influenced by the way in which the malt has been dried. Many of the troubles of the brewer are to be traced to improper treatment at this stage.

Malts may be divided into two distinct types, pale and high-dried; they derive to a large extent their different characteristics during the kilning process. The malts of the former type are used for the production of pale and stock ales, whilst malts of the latter type are reserved for beers of a sweeter and fuller nature, which, as they are rapidly consumed, are technically known as "running ales." Between these two different classes of malt there are a

¹ Messrs. Hanbury recommend in the present state of our knowledge drying on an ordinary kiln floor and curing in a drum.

number of intermediate varieties, partaking more or less of the characters of the one type or of the other.

In all cases the drying of malt is commenced at a temperature preferably below, but not exceeding 100° F., and this should not be exceeded on the first day, care being taken that a certain, but not too large quantity of air passes through the malt. The heat is gradually raised during the second day, until, at its close, the temperature of the malt has risen to 110° or 115° F. On the third day the temperature of the malt will usually be 120° – 130° in the morning, and it is somewhat rapidly raised to 140° – 160° at which it is maintained till the fourth day. During the next twelve hours the heat is gradually raised to the temperature at which it is desired to finish the malt. This will vary from 190° to 230° F., according the character of the malt being manufactured; it is kept at this point for six hours before being taken off the kiln. This, which is generally known as the "curing stage," is a most important portion of the operation, and requires great attention. It must be borne in mind that these temperatures will have to be varied according to the construction of the kiln, the amount of draught, &c.

The object of the very slow application of heat together with a current of air during the earlier stages of the drying process is to remove the superfluous water, and to effect this object without either destroying too much of the diastase or rendering the malt hard and steely. Diastase in the moist condition is extremely sensitive to heat, but when perfectly dry it will bear exposure to the comparatively high temperatures necessary for conferring those desirable qualities which are imparted to the malt during the curing stage. If the green malt is exposed to too high a temperature at first, the starch in the external layers of the endosperm becomes gelatinised, and dries into a horny mass, which effectually prevents the proper escape of moisture from the interior of the grain.

Changes Effected during the Kilning Process.—During the first stages of the drying process the green malt loses from 25 to 30 per cent. of water; it then becomes what is known as "hand dry." This is accompanied with a considerable shrinking in volume. During this period changes similar to those which are brought about by germination still continue to a limited extent. Thus Prior found that the diastatic power in a malt had increased from 88.9 to 134, and that the reducing sugars had similarly increased from 12.58 to 13.74 per cent. The total acidity also increased, in one sample from 0.60 to 0.74 per cent., in another from 0.56 to 0.64 per cent. (reckoned as lactic acid) owing to an increase in the fixed organic acids and in the primary phosphates. As the drying proceeds the remainder of the water is lost, but a series of changes take place which are largely governed by the amount of water which the malt contains at the earlier part of this period. When, as in pale malt, this is small, all those changes of a vegetative nature which had hitherto been taking place are arrested, and the malt gradually loses its raw flavour, but never acquires that highly aromatic flavour characteristic of the highly dried malts. As a very considerable portion has been removed at a comparatively low temperature, the diastase is not seriously impaired; and since during the subsequent

stages little moisture is present in the malt, and as dry diastase is not affected by heat to nearly such a great extent as it is when in a moist condition, the diastatic powers of such malts are fairly high at the finish. But when, on the other hand, the heat is permitted to rise to temperatures towards 160° F. while the malt still contains from 12 to 15 per cent. of water, then the formation of those products on which the aroma of malt depends commences, and the more this stage is prolonged the greater is their amount. Colour is also produced if the malt be subjected to the temperature just mentioned in a moist state, and moreover the colour is of a redder tint than that produced by heating the malt in a drier state at higher temperatures. Further, the diastase is crippled to a greater extent when the malt is heated in presence of water, and it would seem from some unpublished experiments of Ling and Davis that the enzyme is modified in the same manner as it is when heated above 140° F. in aqueous solution. The practice of stewing malt is to be condemned. According to Thausing,¹ the formation of the aromatic principles commences at a temperature of about 150° to 170° F., and it is impossible to produce aroma in a malt when its moisture has fallen below 7 or 8 per cent. By keeping these principles in mind the maltster has the power of varying the character of his malt, as regards flavour, as he chooses. The contents of the corns become browner in colour according to the degree of heat to which the malt has been subjected in this middle stage. This deepening in colour has been attributed by various observers to the alteration of very different constituents of the malt, but its formation depends, to a certain extent, upon the alteration in the proteid constituents, as was first suggested by Habich. This is also borne out by the experiments of Sykes, who found that the albumoses derived from high-dried malt were always considerably darker in colour than those derived from pale malt. Caramel is evidently produced, to some slight extent, probably from the lævulose, the amount of which may be increased during the drying process by the hydrolysis of cane sugar by the acids of the malt (compare, however, below). During the drying the diastase loses much of its activity, and this is advantageous, for, if such were not the case, it would be impossible to brew full-bodied beers. The diastatic power of green malt diminishes from 120° to 100° on Lintner's scale to the average of 45° to 30° under the ordinary conditions of drying; but with careless manipulation it may sink much lower than the latter figure, or even be nearly destroyed.

When malt is allowed to sweat either on the floors or on the kiln it is known to give unfavourable results in brewing and such malt is usually spoken of as "forced." It has been pointed out by Moritz and Morris that one of the characteristics of "forced" malt is the presence of excessive amounts of sugars and soluble non-coagulable nitrogenous compounds, and this has been amply proved by the experiments of others. When malt is heaped up and allowed to sweat the ordinary respiratory changes are arrested and the grain becomes asphyxiated. The starch and albuminoids are attacked by the enzymes and

¹ *Malzber. u. Bierfabrik.*, p. 380.

broken down. As we have already seen, a certain amount of this enzymatic action is necessary for mellowing the malt, but it must occur generally throughout the corn. Some interesting observations on kilning have been made by Grüss, which have a special bearing on the sugars of malt. He divides the kilning process into four stages. The first is that at which the temperature limit is 113° F.; it is characterised by a continuation of the living processes within the corn, especially growth of the acrospire. He points out that if a temperature of 100° F. obtains while the malt carries a large percentage of water, rapid growth of the acrospire occurs, whilst the rootlet dies away. This energetic growth is accompanied by hydrolysis of the starch of the endosperm, the reducing sugar formed being taken up by the aleurone cells and converted into cane sugar; starch is never formed however in the aleurone cells. At the same time another portion of the reducing sugar is absorbed by the scutellum and likewise converted into cane sugar. The cane sugar is in part stored up as such and in part converted into starch. There are therefore two storehouses in the corn for the plantlet to draw upon, the first being the endosperm and the second the scutellum. When, however, sugar wanders from one part of the corn to another, chemical transformations occur. The reducing sugar is converted into cane sugar and the latter into starch, which is reconverted into cane sugar, and as such forms food for the growing plantlet.

The second stage of the kilning process, according to Grüss, is that in which the temperatures range from 113° to 167° F. In this stage the life of the corn is suspended but the enzymatic processes continue. The starch is further saccharified. The dividing partition of the aleurone layer at the furrow is attacked, as are also the cell walls of the endosperm which are still intact, these being converted into gummy substances. If, however, the water is quickly removed from the malt, the last-named gummy substances are not formed. The cell walls of the dividing partition remain intact and the gases within the corn expand at the high temperature, and in this way greatly assist in producing tender malt. On the other hand, in the case of hard vitreous malt, the central portion of the endosperm is filled up with a glassy mass originating from the cell walls. Grüss has recognised another kind of vitreousness due to excess of proteid matter. The transverse section of a malt corn having this fault shows an amber yellow, at times darker ring, having a white central portion.

In the third stage of the kilning process, the oxidising actions associated with ordinary diastase are arrested, but an enzyme which Grüss claims to have recognised and which he denotes "spermoxidase" remains active. It is situated in the plantlet and is also formed in raw barley. The fourth stage of kilning is the curing. These statements are the outcome of direct observation, the various actions having been localised by the examination of sections of germinating barley under the microscope.

Malting Fuel.—The fuel employed by the maltster is either anthracite or coke. The greatest care is necessary in the proper selection of fuel on account of its liability to contaminate the malt with arsenic. In Germany malt is as

a rule dried and cured with hot air, whilst in Great Britain the products of combustion of the fuel are passed through the malt. There is, in fact, a widespread belief among brewers that the fumes arising from the fuel impart a desirable flavour to the malt. The chief source of arsenic in coal is arsenical pyrites the so-called "coal brasses," and shale. The greater proportion of the former may be picked out of anthracite, but even with the most careful selection (which is absolutely essential) it is impossible to guarantee that the fuel is fit for the maltster's use unless it has been analysed. All coal contains arsenic, but for malting purposes the total arsenic should not exceed one-twentieth grain per pound. It is usual to report the proportion of volatile and fixed arsenic, the latter being that which is retained by the ashes, but it is undoubtedly safer to base judgment on the total arsenic. In former times gas coke (which is frequently highly charged with arsenic) was used by maltsters. The best oven coke often contains so little arsenic that it may be used with perfect safety, especially if the precautions to be mentioned later are taken. It is absolutely essential, however, to control the purity of all malting fuel by analysis.

Early in 1901 a patent was taken out by B. E. R. Newlands for preventing the arsenical contamination of malt, hops, &c., by mixing a proportion of lime or other base with the fuel. Coke by reason of its absorbent nature specially lends itself to this treatment. Ling and Newlands¹ and J. L. Baker cite some very successful practical experiments in which malt was dried and cured with gas coke treated with lime. From a very extensive experience one of the authors (A. R. Ling) can state that the best results are obtained when 5 per cent. of lime is used and the coke mixed with it in the form of a sludge. Successful as this process has been, it is not advisable to employ fuel containing a large proportion of arsenic (gas coke, for example), nor is it safe to omit the analysis of fuel. E. S. Beaven in 1903 obtained a patent for the purification of the kiln gases by scrubbing them through a filter of lime.

According to the recommendations of the Royal Commission on Arsenical Poisoning, beer should not contain more than one one-hundredth grain of arsenic per gallon, and in order to ensure this the malt must not contain more than one three-hundredth grain of arsenic per pound, whilst the other materials must be proportionately pure. As a matter of fact at the present time so great precautions are taken that the material turned out by the best malting firms seldom contains more than one five-hundredth grain per pound.

The proportion of fuel used for drying and curing malt varies according to the quality of the fuel and the construction of the kiln, but on an average it may be put at 50 to 80 lb. per quarter of malt.

Heaping Up.—After the malt has passed through the curing stage it is generally heaped up for a few hours; this is also said to increase its aroma.

Removal of Rootlets.—After the completion of the drying process, the rootlets, technically known as the "coombs," are removed from the malt. This was formerly effected by workmen, who wore heavy boots for that purpose, treading the malt. At the present time, however, the rootlets are removed

¹ *Journ. Fed. Inst. of Brewing*, 1901, 7, 323-325 and 328.

exclusively by passing the malt over a screen. After this the malt is dressed by special mechanical means. It is the custom of some maltsters to store malt with the rootlets still attached, but this is an objectionable practice, since malt coombs have a much more powerful attraction for water than the malt itself.

Storage.—In no other department of malting or brewing has such short-sighted policy been pursued as in the storage of malt. The maltster or the brewer, after all the trouble he has taken to remove, as far as possible, the last traces of water from the malt, and well knowing its extremely hygroscopic nature, often leaves it simply lying in heaps on the floor of the store-room ; or, at best, merely covered with a layer of coombs. Malt, to preserve its good qualities intact, should be stored in bins, which are made as nearly air-tight as possible ; these may be constructed of wood, and lined with sheet iron or zinc, or air-tight rooms may be built of brick, and lined with cement, as are the malt silos so frequently seen in Continental malthouses. According to Brand, malt should never be stored while hot, especially if in large masses ; for, under such circumstances, it gathers heat, its good qualities become depreciated, and it is liable to become rancid. A slight oxidation process, which leads to the formation of carbon dioxide and water, takes place in malt when first stored, and in this way the malt acquires a slightly higher percentage of water. This oxidation has much to do with the “mellowing” which is brought about in the malt during the first few weeks of its storage ; it is well known that malt does not yield good results in the mash tun before it has undergone this mellowing process. Malt should be stored at an even temperature ; if one portion of the mass is warm and another portion cold, the moisture distils from the warmer portion and condenses in the colder ; consequently there is a tendency in the latter portion to become slack, and in extreme cases even to become mouldy. In spite of all the best conditions of storage, malt depreciates in quality after a time ; it is probably at its best two or three months after being made. This is a point which tells strongly in favour of pneumatic maltings, in which malt can be made all the year round.

Chemical Examination of Barley.—In addition to a physical examination, barley is often subjected to a chemical analysis as well. In this way the amounts of moisture, of starch, of nitrogenous matters, soluble and insoluble, and of ash which a given sample contains are determined, and also its degree of acidity.

Moisture.—The amount of water contained in barley generally ranges from 12 to 18 per cent., the average being 15 per cent. Any sample which contains more than 20 per cent. would be considered damp.

Starch.—This is by far the most important constituent of barley, for on the amount of starch which a given sample contains chiefly depends the amount of extract it will yield. Barley contains, as a rule, from 60 to 65 per cent. of its weight of starch ; the thinner varieties, however, contain less.

Nitrogenous Matters.—Barley contains from 8 to 15 per cent. of nitrogenous matter, the average being about 10 per cent. Part of this is soluble in water, but by far the larger part (80 to 85 per cent.) is insoluble.

Some of the soluble nitrogenous constituents are coagulable by heat; these should, according to Kukla, amount to about one-half of the total soluble nitrogenous matter. Barleys which contain lower proportions of coagulable proteids than this require to be worked slowly on the floor in order to secure perfect modification. Samples containing a large total amount of nitrogenous matter were considered at one time to be unfitted for malting, it being thought that such grain invariably yielded hard, steely malts. It is now pretty generally conceded that it is not the absolute amount of nitrogenous matter, but the state in which it exists in the barley, which is of paramount importance. The amide bodies are contained in very small quantities in barley; Maercker and Farsky found the amounts contained in two barleys to be 0.275 and 0.169 per cent. respectively. The ammonia amounted to 0.054 and 0.012 per cent. in the same samples; this latter body is therefore contained in still smaller quantities (compare Appendix).

Acidity.—An aqueous extract of barley is always more or less acid, and this acidity was formerly attributed solely to the presence of lactic acid, hence the custom which still prevails of stating the amount of acid contained in barley, malt, or wort in percentages of lactic acid. According to Prior¹ the acidity of barley mainly depends on the presence of the primary magnesium, calcium, and potassium phosphates, and to a less extent on that of fixed and volatile organic acids. He states that the respective proportions of these bodies in two samples of barley, expressed in percentages of lactic acid on the water-free substance, were as follows:

	I.	II.
Volatile organic acids	0.07	0.05
Fixed organic acids	0.06	0.05
Primary phosphates	0.29	0.25
Total	0.42	0.35

Mineral Constituents.—Barley, on incineration, leaves behind from 2.5 to 3.0 per cent. of ash, reckoned on the dry substance. According to Wolff, the ash of barley consists of the following substances, expressed as percentages on the ash:

Potash	20.92
Soda	2.39
Lime	2.64
Magnesia	8.83
Iron oxide	1.19
Phosphoric acid	35.10
Sulphuric acid	1.80
Silica	25.91
Chlorine	1.02
Total	99.80

The preponderating bases are potash and magnesia, and the predominating acids phosphoric and silicic. The potassium and magnesium phosphates are

¹ *Chem. u. Phys. d. Malz, u. d. Bier.*, p. 40.

the most important constituents of the ash, since they serve as mineral food for the yeast.

Different Kinds of Malt.—Malt is divided into several classes according to its colour; thus we have white, pale, high-dried and amber malt; crystal and imperial; brown and black. In the first four of these the requisite modification is produced by variations in the method of drying. In the manufacture of white malt, comparatively little of which is now made, the best and palest barleys are employed; these are worked with great care and dried at a low temperature. Pale malt is also made from superior barleys, but dried at a higher temperature than white malt. For high-dried malt, lower grade (though not unsound) barley may be employed; the curing takes place at a considerably higher temperature than with pale malt. Amber malt is made from similar corn; it is somewhat more highly dried than the last, and a quantity of wood is sometimes thrown on the fire towards the end of the process; this is seldom done at the present time. Crystal malt is dried in an entirely different manner. After being grown and withered it is placed in a wire cylinder which is rotated over a fire, consequently the grain is dried at a very high temperature. Imperial malt is made in a similar manner to amber, the heat of the kiln being allowed at the finish to rise from 240° to 270° F., oak or beech wood being used as fuel. For brown or blown malt, used for making stout, the barley is germinated in the usual manner, but not allowed the usual length of time on the withering floor. It is then placed on the drying kiln in a layer not exceeding an inch and a half in thickness. Moderate heat is applied at first until much of the moisture has been driven out, when the heat, which is obtained from burning wood, either oak or beech, is suddenly and intensely augmented. Under the sudden application of the heat the grains swell some 25 per cent., and the malt becomes imbued with a strong empyreumatic odour, derived from the products of combustion.

Black or Roasted Malt.—This is prepared by roasting malt in a cylinder, constructed something after the fashion of those in which coffee is roasted. Though well-made malt yields a black malt of a superior character both in point of colour and flavour, yet inferior malts are often used for this purpose, and at times even raw barley. A good sample of black malt is of a uniform chocolate colour, and should not be black. The individual grains should be distinct and clean; if matted and run together and the corn burst, neither the colour nor flavour will be good. Black malt is of an exceedingly hygroscopic nature; no large stock of it, therefore, should be kept in hand. It is best prepared the day before being used, but this entails the necessity of having a roasting apparatus on the premises.

Quality of Malt.—In estimating the quality of a malt we depend upon certain physical and external indications, combined, if possible, with a chemical examination. The first of these enables us to determine the characteristics which it has derived from the barley employed in its manufacture, and also several other conditions induced during the process of malting.

Physical Examination of Malt.—Some indication of the amount of modification which has taken place in the endosperm is ascertained by noting the length to which the acrospire has grown up the back of the corn. Those corns in which the acrospire has not started at all were evidently dead; they are termed “idlers.” Growth should be regular; in a given sample all the acrospires should be grown pretty nearly to the same length. The degree of growth of the acrospire is stated in terms of the length of corn; thus, if it is half the length of the corn, it is said to be half-way up; if three-quarters the length, three-quarters up; and if it extend beyond the end of the corn, it is said to be grown out. If the flooring has been properly conducted, proper modification of the endosperm will be secured when the acrospire extends from two-thirds to three-quarters up the back of the grain, but the maltster must always rely upon the condition of the endosperm itself rather than upon the length of the acrospire. To detect regularity in growth a number of corns are taken promiscuously from a sample, say several lots of 200 each, and the number of corns in which the acrospire has not started or in which it has grown to the various different lengths or grown out are determined by inspection. The different groups of figures so obtained are calculated to percentages.

Thus in three samples the following results were obtained:

Description of Growth.	Idlers.	$\frac{1}{2}$	$\frac{2}{3}$	$\frac{3}{4}$	Fully grown and grown out.	Damaged Corns.
No. 1	2%	37%	30%	23%	5%	3%
No. 2	0%	5%	15%	75%	3%	2%
No. 3	1%	6%	30%	60%	2%	1%

Of these three samples, No. 1 exhibits irregularity of growth, whilst Nos. 2 and 3 are satisfactory; but as above mentioned other considerations are necessary before an opinion can be pronounced on the quality of the malt. For one thing, it must be remembered that modification depends on the growth of the rootlet as well as on that of the acrospire, and the former cannot be determined in a sample of finished malt.

Condition of the Endosperm.—The contents of the endosperms should be tender and friable; the corns should be crisp, and, when bitten, should crumble between the teeth; a broken corn, when drawn across a board, should leave a mark such as a piece of soft chalk would do. Hard glassy corns indicate that either a bad sample of barley has been malted, or that there has been mismanagement in the drying process. The number of friable, glassy, and medium corns may be ascertained by cutting a number through with the kornprufer used in examining barley, and the percentage of the different classes determined in the same way. If more precise information be required the separate corns should be cut longitudinally and transversely with a sharp pocket knife, or with a special apparatus. Examination by the diaphanoscope (see p. 436) gives the surest results.

The Sinker Test.—This method of examining malt is one of great antiquity. It consists in introducing a known number of corns into water, stirring well, and after about half a minute separating the corns that sink from those that float and counting the number of each. The principle of the method depends upon the fact that those corns in which the starch, or a portion of it, is in a steely or vitreous condition, have a specific gravity above that of water, and consequently sink. It has been condemned by many recent writers as giving misleading results, *vide* Norbert Van Laer.¹ On this point it may be remarked that when a large proportion of the corns in a sample of malt are found to sink, the sample cannot be a good one, but it does not follow that a sample is faultless which contains no sinkers. Meacham² has suggested a modification of the method which he finds to yield results of great practical value. He prefers to speak of the method as the separating test, and he carries it out as follows:—About 300 corns are measured off by means of a suitable vessel (measuring is quicker than counting and only involves a minute error) and thrown into water with which 5 per cent. of alcohol has been mixed, well stirred for about half a minute, and the sunken corns separated and placed on a sheet of blotting paper. The corns are now examined one by one, by cutting with a penknife, and the results recorded according to the scheme shown in the following table :

Date.	Name.	Soft. D.	Hard. D.	V.	S.B.	S.E.	S.T.	Bad Points.

D.—Dead or idle corns ; V.—Vitreous corns ; S. B.—Steely backs ; S. E.—Steely ends ; S. T.—Steely tips ; Bad points ; Aggregate of defectiveness. Meacham gives the different defects marks as follows :—D. (soft), 5 ; D. (hard), 4 ; V., 3 ; S. B., 3 ; S. E., 2 ; S. T., 1.

Reichard carries out the sinker test as follows:—Two lots of 100 corns each are placed in beakers of about 800 cc. capacity and a stream of water directed upon them. The number of sunken corns which assume a vertical, a half vertical, and a horizontal position in the water are at once counted and also the number of floaters assuming a vertical position. The contents of the second beaker are allowed to stand for twenty minutes and the corns then counted as in the other case. In a later paper Reichard states that alcohol of specific gravity 997 is the most convenient medium for the sinker test.

The sinker test when used empirically, that is, apart from scientific considerations, must of necessity frequently prove misleading. It must be remembered that the food which the plantlet needs for its growth is furnished by the breaking down and rendering soluble of certain of the contents of the

¹ *Journ. Fed. Inst. Brewing*, 1898, 4, 300.

² *Ibid.* 1899, 5, 87.

endosperm. The action on the contents of the endosperm should not be too great or too little, neither should it be local—too great on some parts and too little on others. In a good malt the germ food is produced in normal quantity and the majority of the corns have a lower specific gravity than water and consequently float horizontally. A hard undergrown malt of high bushel weight will be under-modified and the corns will for the most part sink and lie horizontally. A forced malt may be over-grown and over-modified. The over-grown corns will, as a rule, at first float vertically with the germ ends uppermost, and then sink because the space at the germ end fills with water; they do not contain an excess of sugars as these have been respired away. On the other hand, the corns in a forced sample in which the growth of the acrospire does not exceed the normal usually contain an excessive amount of sugars. They may be either floaters or sinkers; in the latter case the difference in the specific gravity of the two extremes of the corns is at its maximum, the germ end being often practically empty whilst the distal end is hard and unmodified. Malt of this kind is produced by rapid growth and high flooring temperature.

Percentage of Moisture.—Malt on leaving the kiln should be practically free from moisture—at all events, it should contain less than 1 per cent; it is, however, an extremely hygroscopic substance, and consequently absorbs water with great avidity. When malt has absorbed even small quantities of water it rapidly deteriorates and becomes “slack”; it undergoes a series of unexplained changes which render it in a short time quite unfit for the production of first-class beers; in fact, slack malt is probably one of the most frequent causes of trouble in the brewery. Though it may be improved to a slight extent by re-drying, it never regains its original good qualities. The amount of water contained in a malt is best ascertained by a chemist, who has all the apparatus necessary for such a determination at hand; but considerable information can be gained by the way in which a malt “bites.” In slack malt all the crispness is gone, the contents of the corn are soft, and convey a general impression of staleness, difficult to describe, but easily appreciated by practice. As it is almost certain that the objectionable changes in malt caused by excess of moisture begin before slackness can be determined in the way just described, the only certain method of ascertaining when a malt contains a detrimental quantity of moisture is by an actual moisture determination in the laboratory.

Broken Corns.—These, when they occur in a sample of malt in anything beyond the smallest proportions, exercise a very prejudicial effect upon the beer brewed from it: their presence is wholly undesirable, and in a good malt they should not exceed 2 per cent.

Age.—Malt should not be used until it is about six weeks old. If used when too new, it is believed by many brewers to cause trouble in the clarification of the resulting beer. Malt over four months old, when kept under ordinary storage conditions, and even before then if kept under bad ones, will become slack; it will then exhibit all the ill effects due to this condition. When kept in air-tight rooms or bins it can be preserved without deterioration for con-

siderably longer periods. Much more attention has been paid in recent times to the provision of proper air-tight chambers for the storage of malt, and this has been followed by a marked improvement in the quality of samples which have been stored for a considerable time.

Odour and Flavour.—Good malt has a pleasant aromatic odour; if it possesses a faint and unpleasant, or a fusty, mouldy, sour, or rancid smell, it cannot be used with any degree of confidence. The flavour, which depends very materially on the way in which the drying process has been carried out, is best detected by chewing a few grains; it should not be raw, for that denotes insufficient heat during the curing process, neither should it be excessively sweet; it should be aromatic, the aroma being developed to the greatest extent in those samples which have been submitted to a high temperature for several hours at the end of the kilning process. The aroma is not so marked in pale malts, which are finished off at a lower temperature. A burnt, bitter flavour points to mismanagement in the kilning process.

Size.—It is possible to make equally good malt from either large or small corn, provided it possesses the other requisite qualities; but of two samples, equal in other respects, the one with the larger corns will yield the more extract, since there will be a smaller proportion of husk to contents.

Weight.—Much importance used to be formerly attached to the weight of malt. From 40 to 44 lb. a bushel is the usual weight met with; and undoubtedly heavy malt, provided it possesses the other necessary qualities, yields the best extract. Samples should be carefully examined, in order to ascertain that the malt does not derive this quality from some imperfection, such as steeliness.

Cleanliness.—Malt should not contain gross impurities, such as stones, dirt, fragments of iron, or foreign seeds in any quantity. All these should be removed from the barley before steeping. The surface of the malt is always more or less contaminated with bacterial organisms, mould, and yeast fungi, which were deposited on the barley when growing in the field, and have survived the heat of the drying process. Machinery is now obtainable which, by a combination of screening and brushing, removes a considerable proportion of the adherent dirt, both of an inorganic and organic nature, and this is exceedingly advisable. The proportion of arsenious oxide present in malt (see p. 465) should not exceed $\frac{1}{300}$ th grain per lb. As a rule it is far below this amount. By placing a small quantity of malt in a test-tube containing sterilised water, plugging up the tube with sterilised cotton-wool, and keeping the whole at the ordinary temperature for a few days, a microscopical examination of the liquid will show what a number of the germs of different organisms are deposited on malt. Malt should be free from rootlets;¹ their presence indicates defective screening.

Chemical Examination of Malt.²—This affords exceedingly valuable

¹ A concentrated extract of malt rootlets is frequently added to wort as "yeast food."

² In April 1905 a Committee, consisting of Messrs. L. Briant, A. J. Brown, A. C. Chapman, John Heron, A. R. Ling, E. R. Moritz, A. G. Salamon and L. T. Thorne, was

information, not only as to the way in which the malting process has been conducted, but also as to the way in which the malt will comport itself in the brewery. The report of an analysis of a malt usually gives in percentage terms the moisture contained in the sample, which is the index of its slackness; the amount of extract the malt is capable of yielding, together with the general character of the wort and its tint; the amount and condition of the nitrogenous matters of the malt; the diastatic power; the percentage of cold water extract (consisting of sugars and nitrogenous matters), and the acidity.

Moisture.—In samples directly off the kiln the moisture is usually below 1 per cent., and the limit of moisture in stored malt may be placed at 3 per cent. As soon as it exceeds this limit the malt rapidly becomes “slack” and deteriorates.

Extract.—The amount of extract yielded by different malts varies considerably; fine, plump malt, when of good quality, will naturally yield more extract than thin samples. The amount of extract yielded by malt in this country is always stated in pounds of brewers’ extract per quarter, hence the cost of each pound of extract can be obtained by dividing the price per quarter by the number of pounds of extract yielded. Thus, if a malt yield eighty pounds extract, and cost thirty-seven shillings a quarter, each pound of brewers’ extract costs fivepence-halfpenny per pound. But the quality of the extract must also be taken into account, as it is of more importance than the quantity. Malt yields from seventy-five to ninety-eight pounds extract per quarter, but these yields, which are laboratory values, vary considerably in different seasons. The report of a chemical analysis of a malt, in addition to the amount of extract yielded, contains particulars as to the odour of the wort, the way it filters—bright or cloudy—the way in which it breaks on boiling, and the proportion of matter fermentable during the primary fermentation.

Colour of Wort.—This naturally depends upon the kind of malt, whether it is pale or high-dried, and is generally stated as pale, medium, dark, &c. The tint of a wort of given strength is now usually recorded in colour units by means of Lovibond’s tintometer.

Nitrogenous Constituents.—The total amount of the nitrogen contained in the malt is first ascertained, and the figure thus found multiplied by a factor, generally 6.25, and the quotient given as the total nitrogenous matter of the malt. The factor 6.25 is employed under the erroneous assumption that all the nitrogenous matters of malt contain 16 per cent. of nitrogen, but it is now well known that the amide constituents contain considerably larger percentages of nitrogen. Figures obtained in this way, however, serve for purposes of comparison. The amount of nitrogenous matter soluble in water

appointed by the Council of the Institute of Brewing to report as to the best uniform methods of determining Extract, Tint, Diastatic Power, Ready-formed Sugars, and Moisture in malt. The Report of the Committee is published in the *Journal of the Institute of Brewing* (1905, vol. 12, p. 1).

is then found in a similar manner, and deducted from the total nitrogenous matter found before; this gives the percentage of the insoluble nitrogenous substance. Malt contains a slightly less amount of nitrogenous matter than the barley from which it was made, since a portion of the nitrogenous substances passes into the rootlets, which are removed from the finished malt. During the malting process a large quantity of the insoluble nitrogenous matters present in the barley become converted into soluble modifications. Attempts have been made to subdivide the nitrogenous constituents of malt into various groups by Ullik, Bungener and Fries, and others. The following is a comparative analysis of malt and the barley from which it was made by the two last-named chemists:—

BARLEY.

	Nitrogen. Per cent.	Factor. Per cent.	Per cent.
Nitrogen of total nitrogenous matter .	1.69	× 6.25	= 10.6
Nitrogen of total soluble nitrogenous matter	0.407	× 6.25	= 2.54
Consisting of { albumen . . .	0.161	× 6.25	= 1.00
{ peptones . . .	0.040	× 6.25	= 0.25
{ amides . . .	0.026	× 6.25	= 1.29

MALT.

Nitrogen of total nitrogenous matter .	1.58	× 6.25	= 9.87
Nitrogen of total soluble nitrogenous matter	0.824	× 6.25	= 5.16
Consisting of { albumen . . .	0.230	× 6.25	= 1.44
{ peptones . . .	0.060	× 6.25	= 0.37
{ amides . . .	0.534	× 6.25	= 3.34

The nitrogen figures have been multiplied by the factor 6.25 for purposes of comparison.

The following figures were obtained by W. J. Sykes, some years since from samples of malt from different sources, all of which were yielding good results in the brewery:—

	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Total nitrogenous matter . . .	8.1	9.2	9.4	10.0	10.1	11.8
Soluble „ „ . . .	3.8	3.6	3.9	4.5	4.5	4.1
Coagulable „ „ . . .	0.9	0.7	0.8	0.6	1.7	1.4
Proteoses and peptone . . .	0.7	0.8	0.5	0.9	0.7	0.6
Amide	2.2	2.1	2.6	3.0	2.1	2.1

The amount of the soluble nitrogenous matters was obtained by estimating the nitrogen of a cold-water extract of each malt; this was then reduced to a percentage of the weight of the original malt, and multiplied by the factor 6.25. Another portion of the same extract was boiled, and the amount of matter coagulable on boiling ascertained. The proteoses and peptones were precipitated in the filtrate from the coagulable matters by means of phosphotungstic acid, and the nitrogen in the precipitate estimated. After deducting the amount of the coagulable proteids, proteoses, and peptones from the total nitrogenous matter, the remainder was put down as amides. In a number

of subsequent experiments, where the proteoses were precipitated by saturating the malt-extract with ammonium sulphate, only the smallest traces of peptone could be detected.

Diastatic Power.—The method for ascertaining the diastatic power of malt generally employed is that of C. J. Lintner, and the results are given as degrees on Lintner's scale. Malts vary much in diastatic power, according to the way in which they have been grown, and especially according to that in which they have been dried. As a rule, the longer the acrospire is grown, the more diastatic will be the green malt. Diastase, when in a moist state, is exceedingly sensitive to heat; consequently, if the green malt is submitted to too high a temperature at the earlier stages of the drying process, when it still contains a large quantity of water, the diastase may be almost completely destroyed. During the drying process a considerable portion of the diastatic power of malt is invariably lost, and the higher the heat to which the malt is exposed the greater will be this loss. Consequently pale malts have a higher diastatic power than high-dried ones; in chocolate or black malts the diastase is completely destroyed. Fortunately this partial destruction of the diastatic power is advantageous to the brewer, for malts as ordinarily manufactured show, as a rule, more diastatic power than is required, and consequently this has to be still further reduced in the mash-tun. It is this excess of diastatic power which enables the brewer to use a considerable proportion of unmalted material, such as rice, maize, &c., in the mash-tun. The distiller and the vinegar maker, who require malts that possess the highest diastatic powers it is possible to obtain, employ such as would be, from a brewer's point of view, excessively grown; they are also dried off at as low a heat as possible. Green malt taken straight off the floor or out of the cylinder, and therefore possessing the maximum of diastatic power, is frequently employed in these special branches of the fermentation industries.

The diastatic power of English brewing malt ranges between 20° and 40° ; higher or lower figures than these generally point to some defect in the malting process. The diastatic power of green malt ranges from 110° to 125° , consequently an enormous restriction of diastase takes place during the kilning process.

Modification of the Endosperm.—As has already been observed malts differ considerably in the degree of modification which has taken place in the endosperm, and this may be due either to the nature of the barley from which the malt is made, or to faults in the malting process. Very often the action of the enzymes has not reached to the extreme end of the barley-corns, and in this way malt with "hard ends" is produced. An ingenious method has been proposed by Moritz for ascertaining the amount of modification which has taken place during the malting process. Two experimental mashings are made; in the first the malt is treated for several hours with a cold-water extract of green malt (which is supposed to contain the cellulose-dissolving enzyme); in the second the malt is similarly treated with the same amount of the same malt extract which has been boiled (in order to destroy the

enzyme) and allowed to cool. After heating both to the mashing temperature, the worts are filtered off and their respective gravities ascertained. The difference between the gravities is assumed to afford an indication as to the amount of modification.

Acidity.—The normal proportion of free acid in malt is from 0.2 to 0.3 per cent., expressed in terms of lactic acid, and it should not exceed 0.4 per cent. This method of expression is purely conventional, the acidity is never entirely due to lactic acid, for there are other organic acids present in malt, and part of the acidity is also due to the presence of acid phosphates; however, it suffices for purposes of comparison.

The Ready-Formed Sugars of Malt.—It has been conclusively established that cane sugar and invert sugar are invariably present in malt, but opinions differ as to the presence of dextrin, maltose, and raffinose. Kühnemann seems to have been the first to detect the presence of cane sugar in malt.¹ C. O'Sullivan² found the ready-formed sugars of two barleys and malts to be as follows:—

	Barley I.		Barley II.	
	Before Germination.	After.	Before Germination.	After.
	Per cent.	Per cent.	Per cent.	Per cent.
Cane sugar	0.9	4.5	1.39	4.50
Maltose	—	1.2	—	1.98
Glucose	1.1	3.1	0.62	1.57
Lævulose	—	0.2	—	0.71
Total	2.0	9.0	2.01	8.76

Brown and Morris³ found that the ready-formed sugars of barley and malt were distributed in the following manner:—

	Barley after 48 hours' steep.		Barley after 10 days' germination.	
	Embryos.	Endosperms.	Embryos.	Endosperms.
	Per cent.	Per cent.	Per cent.	Per cent.
Cane sugar	5.4	0.3	24.2	2.2
Invert sugar	1.8	0.2	1.2	2.2
Maltose	—	—	—	4.5
Total	7.2	0.5	25.4	8.9

Jalowetz found in malt, in addition to the previously mentioned sugars, a

¹ *Ber. d. Deutsch. chem. Ges.*, 1875, 8, 202.

² *Journal of the Chemical Society*, 1886, p. 58.

³ *Text-Book of the Science of Brewing*, p. 74.

carbohydrate, which had the same optical rotatory power and reducing power as maltose, but which was unfermentable; he also considered that he had been able to detect the presence of dextrin, but the results of other investigators render this extremely doubtful.

Morris¹ stated that he had found a body in the unfermentable residues of wort and beer which reduces Fehling's solution. This he thought might probably be a product of enzymatic action on the cellulose walls of the starch-containing cells of the embryo of the barley.

Import of the Ready-Formed Sugars.—Moritz and Morris² consider that an excess of the ready-formed sugars, or, what is a better expression, of the ready-formed soluble carbohydrates, point to errors in the malting process, such as forced growth, and also to wet loading on the kiln, and this is undoubtedly the case. According to their views, these bodies should not exceed 16 per cent; malts containing a higher percentage than this are stated to "give bad results in brewing." Abnormally-low percentages (under 10 per cent.) point to insufficient germination. In this way they propose to use the estimation of the ready-formed soluble carbohydrates as a test for the way in which the malting process has been carried out, but the utility of the method was strongly controverted by Chapman and Heron in a discussion which ensued on the reading of a paper on this subject by Morris.³

Ling⁴ showed that when ground malt is digested with water at ordinary temperatures for three hours (the means usually adopted for the extraction of the sugars and other soluble substances from malt), a notable amount of the starch of the malt is hydrolysed, the maltose formed in this way being erroneously included among the ready-formed sugars. To overcome this he suggested extracting the malt with a dilute solution of caustic potash or caustic soda. Dilute ammonia is recommended by the Malt Analysis Committee appointed by the Institute of Brewing. Ling and Rendle⁵ have further developed this method, and they show that the percentage of soluble carbohydrates is higher in a malt which has been forced, *ceteris paribus*, than in one grown on non-forcing lines, but that there are insufficient data for the fixation of a limit, such that a malt containing soluble carbohydrates beyond that limit can be definitely said to be forced. They point out also that forcing is not the only factor which influences the soluble carbohydrates in malt, and that just as there are *à priori* reasons why a forced malt should contain a high percentage of these substances, so also there are similar reasons why the amount of these carbohydrates should be reduced by respiration, aerobic and anaërobic.

Maltol.—This substance was discovered by Brand in caramelised malts; it is chiefly interesting because it gives with ferric chloride a reaction very

¹ *Journ. Fed. Inst. Brewing*, 1895, 1, 125.

² *Text-Book of the Science of Brewing*, p. 165.

³ *Journ. Fed. Inst. Brewing*, 1897, 3, 229, 230.

⁴ *Ibid.*, 1898, 9, 189.

⁵ *Journ. Inst. Brewing*, 1904, 10, 238.

similar to that yielded by salicylic acid, and this might lead to a mistake being made in the analysis of beer or porter for that acid. Maltol does not, however, give the intense red coloration with Millon's reagent which salicylic acid does; hence the two can be readily differentiated. Maltol is a crystalline, colourless, odourless, volatile substance which melts at $159^{\circ}\text{C}.$; it is readily soluble in hot, difficultly soluble in cold water; very soluble in chloroform, fairly so in alcohol and ether, but nearly insoluble in petroleum spirit. It has the formula $\text{C}_6\text{H}_6\text{O}_3$, and exhibits the characters of a weak acid, forming crystalline salts with several of the alkalis and heavy metals. H. Kiliani and M. Balgen¹ bring forward evidence indicating that maltol is a methyl pyromeconic acid.

Malt Substitutes.—Within the last few years many preparations—so-called “malts”—made from rice and maize, have been placed on the market. In these the more or less complete gelatinisation of the starchy contents of the grain has been brought about by the action of moisture and heat. Their chief defect is the presence of certain nitrogenous organic matters and oil; and if used in too great proportion unsound beer having an unpleasant palate is the result. It has been proposed to make malt by growing on barley or other grain a mould fungus, in the same way as the Japanese manufacture koji from rice. Several of the mould fungi secrete diastatic enzymes in great abundance.

¹ *Ber. d. Deutsch. chem. Ges.*, 1894, **27**, 3115.

CHAPTER VIII.

ARRANGEMENT OF THE PLANT IN A BREWERY.

IN giving a general survey of the arrangement of the various vessels used in brewing, the type of brewery built upon what is termed the "tower system" has been chosen, not that it is necessarily the best arrangement possible, but because it is the simplest and most readily understood. It also occupies the smallest superficial area, and this is a matter of importance when the site is limited.

A "tower or gravitation brewery" is so called because one portion of the building is built at a greater elevation than the remainder. The liquor and goods used for the brew are raised to the highest storey of this elevated portion or "tower," and as the operation of brewing proceeds, they gradually descend by gravitation. In this arrangement each vessel is said to "command" the next in order of use, and all intermediate elevation or pumping is avoided.

The accompanying figure (86) illustrates the arrangement of a modern brewery on this system. In the roof of the topmost room of the tower is the cold-liquor back A, and on the floor of the same the hot-liquor back B, also the upper portion of the grist-case C, by the side of which is seen the highest part of the elevator. In the room immediately below are placed the lower part of the grist-case, the mashing-machine, the mash-tun D. On a platform immediately under its floor is the under-back E, and in a room to the side the copper F and the hop-back G. Coming back into the tower proper, we find the cooler H, and immediately below, the refrigerator, and the malt-case J, K. In the lowest room there is the malt-mill L, the lowest portion of the elevator, the engine, and the pumps N, situated over the well. Slightly to the left are the steam-boilers O, which furnish the steam for heating the hot-liquor back, and the copper, and for driving the engine. In the uppermost storey of the portion of the brewery to the right is the storeroom for the malts and hops; on the floor below, the fermenting rounds. The lowermost storey forms the racking-room, and is furnished with the racking tanks PP.

The tower system, though it is admirable so far as the actual process of brewing is concerned, has several disadvantages. It does not permit of extension as the business of the brewery increases; there is, as a rule, no room for further enlargement of plant; and, when this is required, the erection of an

additional tower is necessitated. It is also inconvenient for the brewer to be compelled to frequently visit different floors.

Other plans have consequently been devised to reduce the height of a brewery, though this increases the extent of surface occupied. In these the

FIG. 86.—Schematic View of a Gravitation Brewery.

wort is elevated at one or more points; it may, for instance, be pumped into the copper as it leaves the mash-tun, the copper being placed at the same or a higher level than the mash-tun; or the wort, as it leaves the hop-back, may be pumped into the cooler, which in this case may be situated in the topmost room of the brewery. This latter is the preferable plan, when the wort is only pumped once.

The height of the brewery may be still further reduced, and the superficial area on which it stands increased, by pumping the wort at both these points, and this is the plan most frequently found in old breweries. In times gone by it was thought that any building could be transformed into a brewery, and the result of commencing with inappropriate structures, and afterwards making alterations in them when increase of business has necessitated such a course, is the many complicated and ill-contrived breweries so often met with, where it is impossible to apply those rules of rigid cleanliness which science has taught to be so indispensable in the successful practice of brewing. Fortunately at the present time, from the efforts of a number of architects who have devoted much attention to the construction of breweries and malt-houses, their general arrangement is now being placed on a much more scientific and rational basis.

Cold-Liquor Back.—This is a large rectangular tank, sometimes made of wood, but more often of flanged cast-iron plates, the planed flanges of which are bolted together. It is, as a rule, placed in the highest storey of the brewery, and is occasionally so arranged as to form a portion of the roof. The water from the well is pumped directly into this vessel, which very often acts as a sort of general reservoir for the brewery, both brewing and attemperating water being drawn from it. Such an arrangement can scarcely be recommended; the refrigerating water is much better drawn directly from the well, the water of which is much cooler in summer than that which may have been standing for some hours in the back. The size of the back will depend upon whether its contents are to be used for brewing alone or for both brewing and refrigerating. In the former case, this may be fixed at the rate of $2\frac{1}{2}$ barrels per quarter for the largest number of quarters ever brewed at one time; in the latter, five barrels per quarter will not be too ample an allowance.

Hot-Liquor Back.—This is a covered vessel, generally constructed of iron, which may be either circular or rectangular in shape, and is situated at a higher level than the mash-tun. To prevent loss of heat by radiation, the back should be either lagged with wood or covered with some non-conducting material. Arrangements of various kinds are provided for heating its contents. The simplest plan is an arrangement of perforated pipes that allow the admission of steam into the liquor, but this though very effective, has several drawbacks. For instance, impurities may be carried over with the steam if the boiler is fed with dirty water, or if chemicals are used to prevent boiler incrustation. If the engine used be a condensing one, oil and grease of various descriptions find their way into the boiler along with the feed water, and these may be transported by the steam into the hot-liquor back. Moreover, the steam when entering the liquor, especially when the latter is cold, makes much noise and causes a considerable amount of vibration; contrivances, however, are now made which obviate these latter inconveniences. The most common method of heating the contents of a hot-liquor back is by a coil of copper or iron pipes

placed along the bottom of the back, into which steam is admitted. The steam in condensing gives up its heat to the cold liquor, and escapes as water at the end of the coil. The coil has a steam-trap attached to its farthest extremity, which, whilst keeping up the steam pressure, permits the escape of the condensed water. Instead of a coil, high-pressure heaters may be used. These are cylindrically shaped vessels, closed in top and bottom, through which a series of tubes about two inches in diameter pass; into the space between these and the sides of the cylinder steam under pressure is admitted. These are said to cause a greater circulation in the back during the heating process than the coil does. When the latter is employed, the liquor underneath it is apt to escape the heating process, so that when the outlet pipe is attached to the bottom of the back the first portions of the mash liquor drawn off are not at the required temperature. A thermometer bent at right angles is inserted into the back, into which the bulb projects for a short distance, whilst that portion which carries the scale stands outside and is protected with a case. A chain running over two pulleys, with a float attached to one end and a pointer to the other, extends from the hot-liquor back to a scale in the mashing-room, upon which is indicated by the pointer the quantity of liquor in the back. The cover of the back is either removable or is provided with a manhole; in this way access is afforded to the interior of the back for cleansing purposes, or for the introduction of the materials for hardening the liquor where such are employed.

The hot-liquor back should be large enough to contain the whole of the liquor required for one brew, and this is generally reckoned as six barrels per quarter. It is often made only large enough to contain sufficient liquor for the mash, the remainder of the liquor for the brew being heated whilst the mash is standing on. The former is by far the preferable arrangement, since when the brewing water contains calcium and magnesium carbonates in solution, there is often not sufficient time to effect their removal from the sparge liquor.

The Malt-Mill.—This apparatus consists essentially of a pair of smooth rollers, either of steel or chilled cast-iron, mounted on axles. Motion is communicated by a strap running over a pulley fixed to the end of one of the axles; at the opposite end of the same axle a toothed wheel is fixed, and this gears into a similar toothed wheel fixed on the axle of the other roll, by means of which motion is conveyed to it. The distance between the rolls can be regulated with the utmost precision by two set screws which act on the axle bearings of one of the rolls. Grooved rolls are sometimes employed. These, which cut rather than crush the malt grain, are very effective in their action, and do not powder the malt so much as ordinary rolls. They are, however, easily damaged by stones, nails, &c., in the malt, and as they require a special machine to sharpen them, this necessitates their removal from the brewery to be sharpened, whilst the ordinary rolls can often be refaced on the premises.

A large rectangular receptacle of wood or iron, the lower portion of which

has the shape of an inverted pyramid, is fixed immediately above the malt rolls, and this serves as a receptacle for the malt which is about to be crushed. The lower portion of the hopper terminates in a narrow opening situated some inches above the rolls, in which an iron roller, deeply grooved in the direction of its length, is placed; this slowly rotates when the mill is in action, its object being to secure an even feed to the mill and to distribute the malt equally over the rolls. One side of the hopper is provided with a slide, worked by a rack-and-pinion movement, and by varying the distance between the edge of this slide and the feed-roller, the quantity of malt passing to the rolls in a given time can be regulated. The whole of the moving parts are cased in to prevent loss of malt-dust, suitable doors being provided for obtaining access to the various parts when necessary for cleansing or other purposes. The best position for the malt-mill is directly over the receptacle known as the "grist-case," which holds the ground malt or "grist."

When, owing to the arrangement of the brewery, the grist-case is situated at a higher level than the malt-mill, a hopper is attached to the under side of the malt-mill, the shoot of which communicates with the lower portion of an elevating apparatus commonly known as a "Jacob's ladder." This machine consists of an endless leather band, working over two pulleys, one of which is driven by power. A number of light metallic buckets are fixed on the band at regular intervals; these scoop up the grist, elevate it to the required height, and in turning over the top pulley discharge their contents into a shoot which leads to the grist-case. The whole is closed in to prevent the loss of malt-dust. Should it be required to move the malt in a horizontal direction, a trough, in which an endless screw revolves, is used to effect this object. Since both these latter arrangements are apt to separate the more finely ground portions of the malt from the husk, and as this is decidedly disadvantageous, these appliances should only be used where it is absolutely impossible to have the malt-mill placed directly over the grist-case. For the same reason a long drop from the malt-mill to the grist case is to be avoided.

Malt-Mills in Pairs.—A pair of malt-mills fixed side by side is a very common arrangement, and one to be recommended, as the rolls in one of the mills can be set a little closer than those of the other; by passing the smaller malt through the more closely set mill, more perfect grinding is secured. In this case the malt is passed through a screen, which generally consists of a revolving cylinder provided with three sets of differently-sized apertures. Through the smallest of these the dust, dirt, &c., find their way, and fall into an appropriate hopper and retaining-box; the next set of holes are just large enough for the smaller malt to pass through, and the remainder of the malt passes through a series of larger holes; any large foreign bodies, such as stones, flints, nails, &c., fall out at the end of the cylinder. Sometimes, in addition to this, a set of magnets are so arranged as to remove pieces of iron, such as nails, which are frequently found in malt. All these arrangements are decidedly advantageous, since they prevent injury to the malt-rolls and lessen the

danger of explosion. When hard substances such as stones, flints, &c., pass through the rolls, sparks are often struck off, which are apt to ignite the finely divided malt-dust suspended in the air surrounding the mill and the various receptacles. This, when ignited, explodes much as a mixture of an inflammable gas and air does. Various effective contrivances are now made which cut off all connection between the mill and the various passages and receptacles if the malt-dust becomes ignited.

Speed.—Malt-mills should not be driven at too high a speed, or their action becomes too much of a grinding instead of a crushing nature. A speed of about 180 feet per minute for the periphery of the rolls is the outside limit. This, with rolls 8 inches in diameter, would be ninety revolutions per minute.

Care in Grinding.—As much of the success of the process of brewing depends on the way in which malt is ground, considerable care should be exercised in this operation. The more finely malt is ground the more extract it will yield, this applying more particularly to a steely or badly modified malt. But, unfortunately, if the malt is too finely ground, serious difficulties arise in connection with the drainage from the mash-tun. (Compare, however, pp. 492 and 494.) The aim of the brewer should be just to hit the happy medium, grinding to such a degree of fineness as shall secure him a good yield from his malt, and yet not so fine as to involve himself in subsequent drainage difficulties. Every corn should be crushed, or waste will take place, since corns which escape crushing yield up little or nothing of their constituents in the subsequent mashing. Tender, friable malt, especially when recently dried, grinds very readily. Such malt even when coarsely ground yields up its soluble constituents much more perfectly than inferior material. If the mash be passed through a filter press then the malt must be ground to the finest flour (see p. 492).

As a matter of convenience, malt is generally ground the day before it is to be used, but this length of time should not be exceeded. Grist absorbs moisture with extreme avidity, and hence rapidly deteriorates.

Regular Cleaning.—Malt-mills, hoppers, and all other adjuncts to the grinding of malt, should be regularly cleaned to remove the malt-dust which collects in them. If this is not done regularly, the dust becomes damp, and gradually undergoes changes which render its presence most undesirable in the wort.

The Grist-Case.—This receptacle is situated immediately over the mash-tun; its upper portion is rectangular in form and its lower portion shaped like an inverted pyramid, the sides of which should slope at an angle of about 45° , this inclination being found best for the regular delivery of the grist. It is made either of well-seasoned wood, smoothly planed and with well-fitting joints, or of iron well painted and varnished. Wood is the best material, since, being a worse conductor of heat than iron, the steam rising from the mash-tun is not so liable to condense upon the grist-case, and form little rills of water which often find their way into the inside of the case and its appendages. The exact position of this receptacle will vary according as the mashing is entirely performed by rakes or is effected by a mashing-machine;

in the former case, its apex will be a short distance above the level of the top of the mash-tun, and a little way inside it ; in the latter case, its apex will be a short distance from the side of the tun. A slide fixed at its apex allows, when opened, the contents of the grist-case to fall either directly into the mash-tun or into the mashing-machine. Closely fitting doors should be provided to afford free access to every part of the grist-case for cleansing purposes. It is especially necessary that those portions adjoining the slide should be carefully attended to, since malt-dust moistened by the condensed vapour from the mash-tun is very apt to lodge on those parts.

The Mash-Tun.—This is a cylindrical vessel (Fig. 87), closed at the bottom, of either wood, copper, or iron; in rare cases, of wood lined with copper.

FIG. 87.—Mash-Tun.

When constructed of wood, it tapers slightly towards the top, so that the staves may be tightened by driving down the hoops; when made of iron, its sides are parallel. Iron mash-tuns are lagged with wood round the sides and at the bottom, a space being left between the iron and the lagging, which is filled with some non-conducting material, such as slagwool, asbestos, &c. Mash-tuns are generally provided with thick wooden covers, which for convenience in handling are generally made in two or more portions. Large tuns are often provided with a hood-shaped cover, hung on chains, working over pulleys and counterbalanced by weights, so that the hood can be readily raised or lowered bodily. In every case the cover should be constructed of non-conducting material, so as to retain the heat in the mash-tun as much as possible. This vessel should be placed in a situation as free from draughts as possible, since these tend to carry off its heat, and nothing is worse in practice than what is known as a "cold mash-tun," that is, one which loses heat rapidly.

False Bottom.—At a distance of one and a half to two inches above the real bottom of the mash-tun is situated the false bottom, which may be of copper, iron, or gun-metal, constructed in sections for easy removal. These sections are technically spoken of as “the plates,” and, when in position, should fit closely together, no interspaces being left open. Iron plates are perforated with holes of from one-sixteenth to one-eighth of an inch in diameter, situated at a distance of about one inch from each other, their under sides being countersunk. Copper and gun-metal plates are similarly perforated, or they are slotted, *i.e.*, have fine slits cut through them. Cave’s patent plates are either made of copper one-eighth of an inch or of cast-iron three-eighths of an inch in thickness. Holes of three-eighths of an inch in diameter are bored at a distance of two inches from each other, and in each of these is inserted a thin copper thimble, the top of which is on a level with the upper surface of the plate, and is perforated with nine small holes one-thirty-second of an inch in diameter. They give excellent results in practice, and the contact of the dissimilar metals in the iron plates does not seem to give rise to any difficulties arising from galvanic action.

The plates, when in position, rest upon small wooden or metallic supports fixed to the upper side of the real bottom of the mash-tun, and are firmly secured in their places by bolts and nuts.

Rakes, or Internal Mashing-Machine.—Rakes were invented by Matterface in 1807, and have with little alteration survived to the present day. They consist of a vertical shaft A (Fig. 87), which revolves in the axis of the mash-tun, and may be driven by a bevel wheel either from above or below. When driven from above, the shaft works in a footstep fixed to the centre of the mash-tun bottom; but when driven from below, the shaft passes through a water-tight stuffing-box in the bottom of the mash-tun. About half-way up this shaft is a collar which revolves round the shaft, and which carries the gun-metal bearing that supports one end of a horizontal shaft that carries the rakes, GGG. A small toothed wheel is fixed to the other end of this shaft, and this engages with the circular toothed rack E, which is bolted to the inside of the mash-tun. A toothed bevel wheel, F, fixed on the vertical shaft A, transmits motion to a similar wheel placed on the horizontal shaft. The rakes GGG are fixed at right angles to the shaft D, and are arranged around it in a spiral, so that if the shaft be looked at endways, the rakes appear like the spokes of a wheel. The pair of rakes at the extreme end of each shaft are fixed in a vertical position and do not revolve.

When in action the motion is a somewhat complicated one. As the vertical shaft A turns round, the bevel wheel F moves along with it, and rotates the wheel attached to the end of the horizontal shaft which carries the rakes. The wheel at the other end of the shaft engages with the rack E, and causes the horizontal shaft and the rakes to slowly revolve round the vertical shaft A. Thus the contents of the tun are thoroughly roused in every part. The whole apparatus is generally constructed of iron, more

rarely of gun-metal. In Conron's patent rakes the end of each rake is expanded into a stirrup-shaped form, to the horizontal portion of which a piece of stout sheet india-rubber is attached. When in action these elastic flaps sweep the surface of the plates. Their utility, except in special cases, is extremely doubtful.

External Mashing-Machines.—These were invented to ensure a more equable admixture of the hot liquor and grist than was possible by the methods already mentioned. The first machine of this class was invented by Steel in 1853; it bears his name and is the one most frequently used at the present time. The machine (Fig. 88) consists of a horizontal cylinder of iron or copper, A, from 3 to 6 feet in length and from $8\frac{1}{2}$ to 22 inches in diameter. One end of the cylinder is closed with a cap, having in its centre a stuffing-box, through which passes the shaft B. At the other end is a cap, C, which closes half the aperture and carries the bearing in which the end of

FIG. 88.—External Mashing-Machine (Improved Steel).

the shaft turns. The shaft is revolved by means of the pulley D, and is provided with a series of arms, fixed at right angles. The screw blade, H, is a slight improvement made to the original machine with a view to adding to its efficiency. The grist is admitted to the machine by an opening, F, the supply being regulated by a slide which is opened or closed by a rack and pinion actuated by the wheel G. The hot liquor is admitted through the aperture E, a cock being placed immediately in front of it to regulate the supply. The streams of grist and liquor meet at the end of the cylinder, and during their passage down it are thoroughly and intimately mixed by the revolving screw and arms. The gearing should be so arranged that the shaft makes from 150 to 180 revolutions per minute. The machine is extremely rapid in its action; with one of the larger machines it is possible to mash 200 quarters of malt in twenty minutes.

A number of self-acting mashing-machines are also in use. The first of these was invented by Maitland in 1863, and consists of an external copper cylinder which encloses an inner copper cylinder of smaller diameter, the space between these being closed in at the top and bottom. An aperture at

the side admits the hot liquor to this space, and the grist falls through the internal cylinder. This is perforated with a number of fine holes through which the water rushes, and forms a series of jets which impinge against the stream of falling malt. A cross tube situated at the lower end of the cylinder carries a fairly large jet which throws a stream of liquor directly upwards, forming as it were a small fountain. The grist in its passage through the machine is met by numerous streams of hot liquor which impinge upon it in every direction and a thorough admixture ensues. Maitland's machine is rapid and efficient in its action, and has no moving parts to get out of order. Southby also invented a self-acting machine, in which the grist falls in a thin layer between two horizontal sets of fine jets of hot liquor. This form requires a less head of liquor to work it than the others.

Appliances for Raising the Temperature of the Mash-Tun.—The simplest of these is a pipe from the hot-liquor back terminating in the bottom of the mash-tun, and known as the "underlet." Hot liquor of any required temperature can be admitted by its agency underneath the plates, whence, through the perforations in the false bottom, it finds its way into the tun, with the contents of which it can be mixed by the rakes. Liquor introduced in this way is technically called "piece liquor."

Coil Under False Bottom.—A copper or iron coil is sometimes introduced between the plates and the bottom of the mash-tun, by means of which steam under pressure can be introduced. This has the advantage over the underlet of not diluting the goods, but it is somewhat slow in its action. In order to remedy this defect, the coil may be perforated at its sides with fine holes, to admit steam directly into the mash. The steam, owing to the large amount of latent heat which it contains, acts very quickly, and it is possible in this way to raise the contents of the mash-tun to the temperature of boiling water. Obviously this method can only be employed where the steam is not contaminated with impurities.

Steam-Plough.—The same object can be effected by the steam-plough invented by Cave. It consists of a couple of hollow vessels, each of which is somewhat similar in shape to a plough-share, attached to, and revolving with the rake-shaft. It is placed close to the false bottom and just clears the rakes as it revolves. Steam or hot or cold liquor may be circulated at will through the ploughs; hence the mash can be either heated or cooled down to any desired temperature.

The Sparge.—This apparatus, shown in the accompanying illustration (Fig. 89), consists of a cylindrical copper vessel, technically called "the basin," so arranged as to revolve easily round its axis. This is effected either by mounting the basin on a pivot, or, as shown in the engraving, by means of friction wheels, bearing on a small platform attached to the shaft which revolves the rakes, or it may be attached directly to the end of the sparge liquor-pipe by means of a water-tight gland. A pipe communicating with the hot-liquor tank, and provided with a tap, terminates just over the basin of the sparge; this supplies the necessary sparge liquor. The sparge arms

(generally either two or three in number) are attached near the bottom of the basin. These are perforated on one side by a series of fine holes, which should be so arranged that the sparge liquor is distributed equally over the superficial area of the mash. The arms can be removed, and are provided with removable caps at their ends, for the purpose of cleansing. As the perforations are very liable to become stopped, the sparge requires frequent attention; a sparge which works inefficiently is often the cause of loss of extract.

In order to ascertain that the sparge is delivering the liquor uniformly, a wedge-shaped trough, a few inches deep and made of sheet metal, is employed, its length being half the diameter of the mash-tun, and its shape a sector of a circle of a similar diameter. The large end may be about two feet wide. At equal distances of about six inches each from the pointed end, a series of partitions are fixed; these conform in shape with the circumferences of a series of circles having for their radii the distance from the pointed end of the trough to each partition. The spaces between these partitions constitute

FIG. 89.—The Sparge.

so many separate compartments. The apparatus having been placed on the plates in the mash-tun, the sparge is set in action for a short time. It is then stopped, and the level of the water in the different compartments observed. If the sparge is working uniformly, the water in all these will be at the same level; if not, the holes in the sparge immediately above any compartment in which it is at a lower level should be slightly opened, the operation being repeated until all compartments are equally filled in the same time.

The principle of the action of the sparge is the same as that of the well-known Barker's mill; the liquid in the sparge arms tends to exert an equal pressure in all directions, but as it is permitted to escape at one side of the arm, a reaction is set up at the opposite side, and this drives the sparge round.

W. P. Harris and E. J. Guest have devised and patented a new form of sparger (English Patent, 28,432, of 1903). They point out that in the apparatus heretofore employed, the flow of water through the arms is controlled by one cock, so that when a small quantity of water is required to be delivered, the pressure of the water and the speed at which the arms revolve are correspondingly reduced; under these circumstances the water

flows but slowly or dribbles in small streams on to the goods. This is obviated in the new apparatus by the provision of arrangements whereby the flow of water to each of the sparge arms is controlled by a separate cock. The flow through one or more of the arms can therefore be cut off entirely without in any way interfering with that through the remaining arm or arms. The result of this is that when a smaller quantity of sparge liquor is required, it can be delivered at its full force, and the rate of revolution of the arms is not diminished. Another form of sparger has since been patented by W. P. Harris (English Patent, 24,789, 1905). It is provided with a central body constructed as a water motor or turbine, by which the perforated sparge arms are rotated. By the use of this device, the pressure of water is absorbed before it enters the sparge arms, and the liquor is delivered on to the goods in the form of a fine spray so lightly that it does not eliminate their contained air, interfere with their buoyancy, or cause their depression.¹ In order to understand the construction of these spargers, the reader is referred to the patent specifications (*loc. cit.*). The main object of the inventions is to render possible in practice the use of finely crushed grist, whereby the highest possible extract returns are secured. Evidence that this object has been attained is given on p. 494.

The Filter Press.—This apparatus is well known to brewers as it has long been employed for pressing yeast. It consists of a series of frames screwed tightly together, over each of which is stretched a filter cloth; each frame is separated from the next by a plate, so that a compartment is formed between it and the filter cloth. The whole system is so arranged that air pressure can

¹ The theoretical efficiency of a turbine sparger such as that above described may be shown by the following considerations:—By a law of hydrodynamics, the velocity with which water issues from an orifice may be determined by the formula $V = \sqrt{2gh}$, where g is the acceleration due to gravity and h , the head of water. When the orifice is revolving round a centre, the velocity of the water relative to the orifice is:— $V = \sqrt{2gh + a^2r^2}$, where a is the angular velocity and r the distance of the orifice from the centre of revolution. The rate of revolution of the orifice is obviously ar in the opposite direction, and the absolute velocity of the water is therefore:— $V_0 = \sqrt{2gh + a^2r^2} - ar$ where V_0 represents the absolute velocity of the water. If now this formula be analysed, it will be found that the term $2gh$ is due to the static head, whilst the term a^2r^2 is due to the centrifugal head. In a sparger the two ideals to be aimed at are, (1) The distribution of the water over the goods as lightly as possible, so that the spray of water shall have no velocity but simply drop on by its own weight. (2) The distribution of the water evenly so that equal areas of goods receive equal amounts of water. Putting (1) algebraically the condition is:— $V_0 = 0$, that is $\sqrt{2gh + a^2r^2} - ar = 0$ which is only satisfied when $h = 0$. In other words the effect of the head of water must be eliminated from the sparge arms, which should be accomplished in the turbine sparger. Since in this sparger the holes are of constant area, it is obvious that velocity may be synonymous with quantity. Hence at any hole the quantity of water issuing is $Q = ar$. Since ar is the velocity of the water relative to the arm when the effect of head has been eliminated, it follows that Q is proportional to r , a being constant all along the arm. But the area of an annular surface of constant width is also proportional to r . Hence it follows that the quantity of water issuing from any hole is proportional to the area it covers; in other words an equal distribution of holes along the arms means an even distribution of water over the goods.

be applied to assist filtration; the filtrate is run off through taps, one of which is provided for each compartment. A general view of the apparatus is shown in Fig. 90.

The Filter Mash.—The utilisation of the filter press in place of the mash-tun for separating the wort from the goods, has recently attracted a great deal of attention from brewers. The process now known as the Meura Brewing Process is said to have been first successfully applied in a small brewery at Loochristi, near Ghent, by M. Meura, who patented it (English Patent, 16,032 of 1901).

For mash filtration there are several filter presses on the market, but the ordinary form, with but slight alteration, serves the purpose very well. A form of filter press specially designed for brewing in this country has been patented by J. G. Crossman, head brewer at Benskin's Brewery, Watford (English Patent, 8392 of 1902).

The credit of having proved that the Meura process could be used for English brewing belongs to G. A. Seaward, formerly of Benskin's Brewery, Bishop's Stortford. Mr. Seaward and his colleague, Mr. H. L. Grimston, in conjunction with Mr. J. G. Crossman, may be regarded as the pioneers of the process in this country.

One of the authors (A. R. Ling) has on several occasions witnessed the filter mash process through the kindness of the above-mentioned gentlemen. At the Watford brewery, the press is used only for the preparation of a raw grain mash. Grits are heated in a converter with water under pressure and the product treated with a small proportion of malt at a suitable temperature in another vessel. After the requisite stand, the product is passed through the press. A perfectly bright liquid containing the whole of the available matter from the grits is thus obtained.

At the Bishop's Stortford brewery, the process is employed for the entire mash, and a Crossman filter in conjunction with a Billing's converter is there installed. When raw grain is used this is first raised to a suitable temperature to gelatinise the starch, after which a proportion of malt is added. The conversion completed, the main quantity of malt is introduced into the converter, together with the necessary amount of liquor, and mashed at the desired heat. After the requisite stand, the mash is passed through the press, the size of which is adjusted to the number of quarters mashed, so that the grains just fill the different compartments. The grains are finally washed in the press by passing water through them at a suitable temperature.

It is an essential point in the Meura process that the malt be ground to the finest flour, otherwise it has a tendency to clog in the press during filtration. This fine grinding, however, constitutes one of the foremost advantages of the process, for it renders possible the more complete extraction of the malt. Indeed, the yield of extract amounts practically to the highest available. In the ordinary process of mashing, as explained elsewhere, too fine a grist cannot be used on account of the danger of flooding (compare however p. 494). When coarse grist is used in the ordinary process, any

FIG. 90.—Filter Press.

portion of the contents of the corns, which remains unmodified by the malting process (and this is more or less the case of every sample of malt at the distal end), is unattacked, so that much starch, amounting to from 5 to 12 per cent. of the total, remains in the grains. None of this starch is recovered during sparging, and any that does gain access to the wort is in an unconverted state, and is a disadvantage rather than an advantage, as it brings about turbidity. Moreover, the soluble matters extracted by sparging in the ordinary process are for the most part objectionable substances which, unless accompanied by starch conversion products, had better be kept out of the wort. The results obtained at Bishop's Stortford leave nothing to be desired from the practical point of view, and an examination of the grains from time to time has shown them to be practically free from starch. The following values were obtained with samples of first wort, final washings and grains from an ordinary brew of running ale carried out at Bishop's Stortford.

First Wort.

Specific Gravity		1078.2
Maltose (apparent) expressed on 100 parts of wort solids	.	68.4 per cent.
Dextrin	" " " " " "	15.5 per cent.

Final Washings.

Specific Gravity		1003.6
Maltose (apparent) expressed on 100 parts of wort solids	.	58.0 per cent.
Dextrin	" " " " " "	2.0 per cent.

Grains.

Moisture		74.2 per cent.
Soluble Matter		1.9 per cent.
Starch		practically nil.

The Meura process has been studied by numerous experts, including M. Meura, W. Windisch, H. Johnson, and M. J. Cannon and H. Brown. The two last-named authors give a series of elaborate analyses of worts obtained by it and by the ordinary mashing process. Their conclusions are in every respect favourable to the process.

The best evidence in favour of the process is, however, that given by Messrs. Seaward and Grimston, who after two years' daily experience with it, express themselves satisfied that the beers are in no way inferior to those obtained by the ordinary process, but if anything superior.

When a filter press is employed in mashing, not only is the yield of extract raised, but in addition to this there is a considerable saving of time, as compared with the ordinary mashing process. Further, there is no difficulty in collecting the whole of the available extract as a wort of 33 or 34 lb. gravity, and this is a considerable advantage in brewing strong ales, where by the ordinary process it is often necessary to keep back the weaker wort for a party gyle, or hold it over for another brew.

W. Stanley Smith¹ has made a detailed study of brewery extracts, and he shows that in order to obtain the maximum extract fine grinding is essential.

¹ *Journ. Inst. Brewing*, 1906, 12, 229, 572.

He advocates the use of chilled cast-iron, instead of the usual mild steel, for malt-mill rolls, and he points out that when one pair of rolls is fitted on a yielding bed, it will be driven by reason of its hardness, apart from its neighbour and allow any foreign bodies, such as nails and stones, which may have escaped the screens and magnets, to pass through uncrushed; these bodies, however, will be seldom drawn in to the rapidly revolving mill. Mild steel, on the other hand, is invariably badly scored by foreign bodies, and its surface being less smooth, there is a greater tendency for such bodies to pass through the rolls; and every mark or indentation on the mill-rolls is a factor against the attainment of high extracts. Smith states that the rolls may be set at $\frac{1}{20} - \frac{1}{40}$ inch apart, and that the closer they are set, the higher the extracts secured. He proposes that the various qualities and types of malt forming the grist shall be well mixed before grinding and then passed through a screen which separates all corns below a certain size; these small corns may then be separately ground by passing them through a smaller mill kept specially for the purpose. Smith is fully aware of the fact that with a fine grist, such as he advocates, there is considerable danger of a "dead" or "set" mash (see p. 521). To obviate this danger the mash should, he states, be passed through the Steele's machine at just the rate which ensures efficient admixture of the grist and liquor, and no faster. Further the rakes must be cautiously used, as high speed or excessive application will invariably result in a sodden mash. When fine grist is employed, however, the dimensions of the mash-tun must be so regulated that the mash remains buoyant; and Smith points out that there is an intimate connection between depth of goods, drainage and extract yield. In order to secure the highest extract with the least amount of trouble he finds that the depth of goods in a 16-foot tun, and at the finish of mashing with two barrels per quarter, should be about 65 inches wet dip. During the first quarter of the time it is proposed to occupy in making up the coppers, he finds it is best to allow the goods to sink about four inches, and to regulate this lessening of wet dip carefully throughout the process so as to conform to the conditions which are actually taking place in the mash-tun. From the first moment of setting taps, a gradual decrease in the volume of malt contents is set afoot, and the lowering of the wet dip of the goods does not necessarily mean that there is less liquor in the tun, at any given time, but rather that there is a constantly diminishing amount of malt material. The object to be kept in view is the maintenance of a fairly constant ratio of liquor to malt material, so that the relative proportions of each are kept as uniform as possible throughout the process of extraction. A definite and regular rate in the fall of the goods should be aimed at, as this will assist in enabling the extract to be drawn off without causing any violent shock to the goods. Steady running of the taps and sparging on a definite plan not only maintains the ratio of liquor to malt material, but prevents the subsidence of heavy, partially insoluble bodies, such as acrospires and hard ends; the further these are from the plates, and the more they are diffused, the less detrimentally will they act upon the drainage of the tun. Violent and

erratic sparging, conducted without system, will often, on the other hand, occasion such a rapid flow of liquor through the goods, that the partially insoluble substances will speedily form a layer which is practically impervious to the liquor; but a comparatively shallow mash will assist in counterbalancing the effects of an impervious layer. When the taps stop by reason of defective drainage, it is generally believed that the plates of the false bottom are choked. This, according to Smith, is seldom or never the case, since free drainage can generally be restored by one turn of the rakes, and these do not touch the false bottom; the resistance then is in the tun itself.

As illustrating the beneficial effect of a shallow mash on the drainage of the goods, Smith cites the following practical examples:—In a certain tun 58 quarters of malt were mashed with the object of producing an 18 lb. beer. The depth of goods after mashing was 58 wet inches, and some 6.8 barrels of sparge liquor per quarter (or $6.8 \times 58 = 394.4$ barrels) in all, were necessary for making up the coppers. The goods invariably ran well and free and the extract was also satisfactory. On other occasions, 74 quarters of malt were mashed in the same tun, to produce a beer of 22 lb. gravity, the depth of goods after mashing then amounting to 70 wet inches. With such a mash there was seldom such free running as in the more dilute one previously referred to, the finely ground malt tending rather to resist the liquor in its passage through these deeper goods. In all 444 barrels of liquor were sparged, *i.e.*, 6 barrels per quarter, as against 6.8 barrels over the shallower mash, but extracts were much the same in the two cases, so that the extra 0.8 barrel of liquor overcomes the tendency for comparatively shallow mashes to permit the too rapid flow of liquor, with consequent loss of extract. The ideal depth of goods (see p. 495) approximates in wet inches, according to Smith, to the mean of the two gauges quoted above, *i.e.*, $58 + 70 = 128 \div 2 = 64$ inches.

Smith acknowledges that he found it impossible to adopt the system of fine grinding which he advocates under the old-fashioned conditions of sparging; the goods flooded and set without exception at every mash. By using the sparger devised by Harris and Guest, however (see p. 490), most of the difficulties ceased. The arms of this sparger are separately connected up with the liquor tank, the holes are exceedingly small, and the velocity attained under suitable pressure is very great. Under these circumstances the water, instead of descending in comparatively slow streams, is atomised, and it alights on the goods in almost imponderable drops.

With regard to the extract obtained by his system of mashing and sparging, Smith states that when very fine grist is used, the laboratory and brewery extracts are precisely the same, whereas with coarser grist the results are divergent.

The Copper.—Coppers are of two principal kinds—"fire-coppers," *i.e.*, those which are heated directly by fire, and "steam-coppers," which are heated by steam. Of the former there are several varieties, the simplest being a copper or iron pan placed over a furnace, the flues from which are carried

round the copper nearly to its top. An outlet is provided near the bottom, to which a tube is attached which carries the cock for emptying the copper. This pattern is now nearly obsolete, and is only met with in small or old-fashioned breweries.

Fire-Copper.—The most common form of open fire-copper is that shown in the annexed figure (91), known as the “bench copper.” The lower portion is of the shape of an ordinary pan; at about half-way up it suddenly widens out a few inches, to form what is termed the “bench.” From this the copper is continued upwards with parallel sides. The flues which surround the copper are only carried as far as the bench; consequently the portion above the

FIG. 91.—Fire-Copper.

bench, not being heated, has, to some extent, a cooling action on the wort, which tends to prevent boiling over.

The dome-copper is, as its name implies, covered in with a dome, round which there is a sort of tray, which has an outlet into the body of the copper. At the summit of the dome is a large opening, to which is attached a wide tube $1\frac{1}{2}$ to 2 feet in length. When the copper is boiling, the communication is left open between the tray and the copper, and through this the wort, as it boils out at the wide tube, after pouring over the sides of the dome and falling into the tray, finds its way back into the interior of the copper. When the wort has finished boiling the heat is slackened, the plug fixed in its place, and a second batch of wort may be placed in the tray, which will be heated to some extent before being allowed to pass into the copper. This heated wort is also ready for immediately running into the copper as soon as the other wort is out, to “save the copper,” that is, to prevent the bottom from being burnt.

Coppers are sometimes provided with a strainer for keeping back the hops when the vessel is emptied; but this is not an arrangement which can be

recommended, as the strainer soon becomes more or less blocked, and consequently the copper empties itself very slowly.

Steam Coppers.—These differ considerably in shape from fire-coppers. The bottom is bellied outwards, and is made of considerable thickness to withstand the pressure of the steam (Fig. 92). The sides may be carried straight up, as shown in the figure, or they may slightly taper outwards. Attached to and surrounding the bottom of the copper there is an iron dome, which is somewhat larger in size, so that a cavity of from three or four inches, B, is left between its interior and the bottom of the copper. Into this high-

FIG. 92.—Steam-Copper.

A, copper ; B, steam-jacket ; C, steam-pipe ; D, steam-trap ; E, outlet-valve.

pressure steam is admitted by a cock, C, an air-tap being provided to allow the imprisoned air to escape when steam is turned on. This is a very necessary precaution, since as air is so highly expansible by heat, if its exit were not amply provided for an explosion might occur. A pipe for carrying off the condensed water passes from the bottom of the steam-jacket to the steam trap, D, which latter permits the water to escape but holds back the steam. The wort pipe, E, passes through the centre of the copper bottom. The steam-copper possesses several distinct advantages over the older fire-copper. It can be heated up before the introduction of the wort, and the wort quickly raised to a temperature which puts an end to all further diastatic action. The readiness with which the heat can be applied or arrested is a point very much in its favour. It is also vastly more economical; a fire-copper, according to Professor Schwackhöfer,¹ only utilises about 30 per cent. of the available heat of the fuel consumed, whilst a steam-copper utilises 70 per cent., and thus a saving of more than half the fuel employed is effected.

¹ *All. Anzeiger f. Brau.*, 1896, p. 1209.

Hop-Back.—This vessel is required in all cases where the copper is not provided with a strainer. It may be constructed of wood or of iron, and its shape may be either rectangular or circular. At a short distance from the bottom a false bottom of perforated plates is provided to keep back the hops. The hop-back is placed at a lower level than the copper. A series of drainage pipes proceed from holes distributed at equal distances in the bottom of the back to the cooler, or to the pumps which raise the wort into the cooler. Circular hop-backs are often provided with a sparge something similar to that employed in the mash-tun, through which hot liquor is sparged on the hops to remove the wort held back by them.

Cooler.—This is a large, shallow, rectangular vessel of wood, iron or copper. In former years it was used exclusively for cooling the wort from the temperature it left the copper or hop-back to that at which it was to be pitched with yeast. This, however, necessitated many hours' exposure on the cooler, more especially in the summer months when, in certain cases, it was allowed to remain there for thirty-six hours. Morris has pretty conclusively proved that wort as it leaves the copper at a boiling temperature is sterile, but it will be readily seen that by a long exposure on the cooler it is exposed to contamination by air-borne micro-organisms. In the authors' experience it is never safe to allow the wort to remain on the cooler at a temperature below 140° F. Indeed Briant states¹ that by inoculating wort, at as high a temperature as 165° F., with chaff-dust and maintaining this temperature after inoculation for half an hour, he found active growths of both bacteria (rod forms) and yeasts; and when wort was inoculated even at 175° F. with hay-dust, growth occurred.

Apart from preliminarily cooling the wort to a temperature such that air-borne organisms shall not germinate in it, the most important function of the cooler is to allow the deposition of sludge and to effect the hot-aëration of the wort, which according to certain observers is of importance. Pasteur points out (*Etudes sur la bière*) that "boiled wort, if cooled down suddenly or slowly, but out of contact with air, or shaken up cold in contact with air, is opaque when filtered; whilst the same wort cooled down on the coolers where it has taken a certain quantity of oxygen into combination, generally passes through the filter very bright." Briant² arrived at the following conclusions:—
 "1. Hot aëration takes place mainly at high temperatures, and a temperature of 180 – 190° F. is that most favourable. It is practically negligible at temperatures below 130° F. 2. Wort absorbs oxygen only at its immediate surface, so that a large surface must be exposed. 3. Actual agitation with air is much more effective than surface exposure as an aid to absorption. 4. Hot aëration, whilst it does not increase the amount of matters precipitated, secures their separation in such a form that they easily subside, and give a wort which filters bright without difficulty. 5. Under the influence of hot aëration, certain matters which, originally soluble in the saccharine constituents of wort, become insoluble as fermentation proceeds, do so in a form in which they

¹ *Journ. Inst. Brewing*, 1904, 10, 289.

² *Loc. cit.*

readily separate from the beer, and thus give a clear-racking sample. 6. Considerable increase of colour occurs by hot aëration, due to the absorption of oxygen by hop extractives, maltose, glucose, or lævulose present in the wort, but the colour so acquired disappears almost completely during fermentation.’

The cooler is placed in a room in the brewery to which air can be admitted from all sides, for which purpose the windows are provided with louvre boards. This exposure to a rapid current of air is usually supposed to effect the aëration referred to in the last paragraph; it certainly ensures rapid cooling. In some breweries the coolers are enclosed and the air filtered before it is allowed access to the wort. When contamination occurs on the cooler it is, as a rule, by bacterial organisms, since if the wort be run on to the refrigerator at a temperature exceeding 140° F. wild yeasts should be killed.

Refrigerator.—This apparatus is made in many different forms, that most commonly employed being the vertical one, which, in all its variations, is but a modification of the refrigerator invented by Baudelot over twenty years ago. In its original shape it consists of two upright iron standards between which a number of horizontal circular copper tubes are fixed, having attached to their under sides narrow strips of sheet copper, the lower borders of which are cut zigzag something like the teeth of a saw. The cold liquor is admitted into the lowest tube, from whence it passes to the tube immediately above, and so on, until at last it emerges from the uppermost tube. The wort is introduced into a trough placed at the top of the apparatus and perforated with a series of fine holes. Through these the wort flows on to the first tube in a thin layer; after passing over this tube it falls on to the next, and so on until at last it reaches the trough in which the apparatus stands, from whence a pipe conveys it to the fermenting vessel. The toothed strips attached to the lower side of each of the tubes secure an equal distribution of the wort.

In the more modern varieties of this apparatus the tubes are made oval instead of circular in section, thus providing a larger cooling surface, and are soldered into openings in the uprights, which are made of gun-metal. On the exterior of the uprights are fixed a series of removable caps, which can be removed so that the tubes may be cleaned out periodically, an operation which is frequently necessary when the water used for cooling purposes is at all dirty or contains much carbonate of lime in solution. In another variety (the Laurence) the cooling liquor passes between two sheets of copper worked into a zigzag form. A series of removable caps gives access to the interior of the apparatus for cleaning, which is effected by a peculiar-shaped brush. The whole machine is fixed on trunnions, and can be turned bodily over. In another (Shear’s) the tubes are arranged in the form of a circle, the whole apparatus having the appearance of a vertical cylinder.

Horizontal Refrigerators.—When height is a consideration, a refrigerator of the horizontal pattern must be used. This has the form of a shallow rectangular trough, divided at intervals by a number of partitions, through each of which passes a horizontal tube of rectangular section with rounded corners,

through which the cold liquor passes. Each tube occupies the centre of a division, a small space being left between the under-side of the tube and the bottom of the trough. The wort flows into the first division, passes underneath the horizontal tube, and, on reaching the level of the top of the partition, passes over into the next division, and so on, until, having passed the last division, it escapes into the wort main. The refrigerator is set at a slight inclination, being highest at the end where the wort runs in. Horizontal refrigerators are much more difficult to clean than vertical ones, since those parts through which the wort passes are so much more inaccessible.

The quantity of refrigerating liquor required to cool each barrel of wort varies with the temperature of the wort to be cooled, the temperature of the liquor, &c., from two to six barrels, or even more; horizontal refrigerators are said to consume about twice as much cold water as vertical. In breweries provided with an ice-machine, cold brine is passed through the lowermost half-dozen or so of the tubes of the refrigerator, which are provided with a separate inlet and outlet.

Collecting Vessel.—In some breweries a separate vessel is provided in which the wort, after being cooled on the refrigerator, is collected, but more often the fermenting vats themselves are used as the collecting vessels. Where separate collecting vessels are employed, they are constructed in the same form and of the same materials as the fermenting vats. Where the collecting vessel is provided, the wort must, according to the excise regulation, remain in it for at least twelve hours after collection.

Vessels for Primings, &c.—In breweries where it is the custom to add syrup or wort to the ale when in cask, or in other words to “prime,” a special vessel must be provided for such liquids. In these the excise officer takes the gravity and dip when filled, and afterwards the dip from day to day. Where a solution of caramel is used for colouring purposes, a similar vessel must also be provided for it.

Fermenting Vessels.—Many materials have been employed from time to time in the construction of fermenting vessels, such as glazed bricks and tiles, brick lined with glass plates, wooden vessels lined with lead or copper, enamelled iron plates, &c. None of these have stood the test of experience, and nowadays the material chiefly used is wood, though in some parts of the country stone is extensively employed, and slate is also used to a limited extent. One of the principal points with reference to the material used in the construction of a fermenting vessel is that it should be capable of being worked to a fine smooth surface, and retain this during usage, as such a surface permits of the vessel being readily cleansed. The material used in construction should also be able to bear the heat of boiling water without being liable to fracture, since the destructive action of heat is one of the chief agencies relied on in the brewery for keeping micro-organisms in check.

Fermenting vats are generally made either round or square; occasionally oval. The first of these are technically known as “rounds,” the second as “squares.” Rounds are built of staves held together by iron hoops, and are

as a rule, made of oak. They are open at the top, and are carried up some two or three feet higher than the level of the surface of the wort fermented in them, this extra space being provided for the yeast. On one side, at a distance of a few inches above the wort level, an opening of about thirty inches square is made, to permit of access to the tun. This, during the fermentation, is closed with a number of loose boards fitting into grooves, which serve to retain the yeast.

Squares are constructed of planks some two and a half to three inches in thickness; they are bolted together with iron bolts let into the thicknesses of the wood. They are generally made of fir, sometimes of oak, and in recent times American cedar has been much employed. The sides of the square, like those of a round, are several feet higher than the level of the wort to be fermented in it; one of them is similarly provided with an opening which can be closed with loose boards. Vessels of oak or fir require seasoning before use. This, with oak vessels, is a very simple matter; they only require to be scalded out several times with boiling water. As fir-wood contains a considerable amount of resin, this must be partially removed before the vat is brought into use. Southby recommends that such vessels be first filled with boiling water, which is run off the next day. The vat is then to be whitewashed out with a mixture of $2\frac{1}{2}$ lb. of chloride of lime to each gallon of water. After this coating has remained on for twenty-four hours, the vat is washed out with a mixture of one part of hydrochloric acid (spirit of salt) and four parts of water. It is then washed out several times with boiling water, and finally painted out with an ordinary solution of bisulphite of lime to remove the last traces of chlorine. American cedar has the advantage that it requires no seasoning, but may be used straight away after being well scrubbed out.

In those parts of the country where the stone square system of fermentation is almost exclusively employed, the fermenting vats are constructed for the most part of large slabs of hard impervious stone; but, as material of the right quality is somewhat difficult to obtain, a softer stone is often employed. This is objectionable, since by use its surface becomes, in course of time, rough, and cannot be properly cleansed. Slate is now beginning to take the place of stone in the construction of the Yorkshire squares; it preserves a much better surface during wear.

The stone square (Fig. 93) differs materially from the wood square in having a second square built round it, through which cold or warm water may be passed; this acts as an attemperator. The sides and bottom consist of single slabs of stone, which are fitted together by means of grooves, the joints being rendered water-tight with cement, whilst a number of iron bolts serve to retain the slabs in position. Since the weight of a stone square is very great, it is supported on a pillar of masonry. The water-jacket, C, is constructed in a similar manner by four slabs of stone of such a size that a space of about 2 inches is left between its internal surface and the outside of the square proper, and its sides are 3 or 4 inches lower in height than those of the square itself, so as to allow the attemperating water to run away. The square

proper, *A*, is covered over with another slab, having a circular aperture (the "man-hole") of 18 inches in diameter, which is surrounded with a stone ring some 5 or 6 inches high, on which a stone lid provided with a handle fits. In one of the corners of the covering slab there are two smaller openings situated a few inches from each of the sides and about 3 inches in diameter, provided with brass valves, *E*, to which chains are attached. From the under side of one of the valves a tube, *D*, extends to within a few inches of the bottom of the square; this is technically known as the "organ-pipe." The second valve is used for conveying the yeast at the end of the fermentation

FIG. 93.—Yorkshire Stone Square.

A, the square; *B*, yeast-trough; *C*, water-jacket; *D*, organ-pipe; *E*, valve.

into the vessel which is ready to receive it. Upon the upper side of the covering slab is placed the yeast-trough, *B*, constructed of four stone slabs. It has the same superficial area as the square, and a depth of from 24 to 30 inches. A pump is one of the necessary adjuncts to a stone square. It is of the simplest possible construction, made of tinned copper and of one uniform size (3 inches) in diameter, and with a stroke of 6 inches. When the square has been put together, it is carefully pointed out with cement, so that no crannies are left where yeast or other organic matter may be retained; and the pointing should be inspected every few months, to see that it remains intact, for, if it becomes defective, troubles are certain to make their appearance in the brewery.

Slate squares are generally not provided with a water-jacket, an ordinary attemperater being fitted inside instead. Slate possesses the advantage of

having a smoother surface than stone, but it has the disadvantage of being a better conductor of heat, and consequently the wort in such a square is much more subject to the influence of changes of temperature in the external atmosphere. Slate will not bear the application of boiling water or steam without cracking or splitting, and it cannot, consequently, be sterilised by these agents. It is also readily attacked by sulphurous acid and the acid sulphites, and the only germicide of this nature which can be employed is the neutral sulphite of lime, with which the inside of the square may be whitewashed. A deposit of calcium oxalate is liable to form on its surface, which is somewhat difficult to remove; rubbing with a soft brick or the application of a strong solution of caustic potash or soda form the best methods for removing this.¹

Fermenting squares constructed of iron plates are not unknown; they are only employed in the production of black beers.

Outlets.—In fermenting vats worked on the skimming system, the outlet or outlets must be so arranged that the beer can be drawn off without disturbing the yeast, which invariably deposits to a certain extent on the bottom of the tun. This may be effected by having two cocks, one at the side some 3 or 4 inches above the floor of the tun, from which the clear beer is raked off into the trade casks, the other fixed into the bottom of the vat, and by this the remainder of the beer is drawn off into vessels where it may settle, or into filter-backs. A single outlet may be provided at the bottom of the tun, through which a tube, having the cock at its lower extremity, slides through a stuffing-box. As soon as the beer has been drawn off to the level of the top of the tube, this is gradually lowered little by little as long as the beer runs bright. When yeast begins to appear, the tube is drawn down to its fullest extent, and the thick bottoms treated in the manner just described.

Loose Pieces.—In some breweries where the cleansing system of fermentation is employed, the wort, after having been fermented to a certain stage in the square or round, is run off into a series of casks or puncheons which hold from three to four barrels each. These are provided with the usual bung and tap holes, and are placed on long troughs called "stillions," which catch the yeast that escapes from the bung-hole and runs down the side of the cask. The loose pieces are inclined a little to one side, so that the yeast may run down one side of the piece. In some cases the trade casks—that is, the casks in which the ale is sent out to the customers—are used for this purpose. Under this system the yeast has to run down the side of the cask, and this is obviously objectionable on the score of cleanliness. Various methods have been tried to overcome this faulty arrangement, such, for instance, as the employment of short, conical tinned pipes, which can be inserted water-tight into the bung-hole of each loose piece, and the upper end of which can be closed by a wooden plug. To the side of each conical tube there is fixed at right angles a pipe of about 2 inches in diameter, long enough to project over

¹ For a detailed description of the Yorkshire stone square system of fermentation see J. Watson Lovibond (*Boake's Diary*, 1886); T. A. Glendenning (*Brewing Trade Review*, 1903, 292; *Journ. Inst. Brewing*, 1905, 11, 400).

the end of the piece, and bent at its extreme end to a right angle. In this case the yeast trough is placed in front of the pieces, and may be made to serve for two adjacent and parallel sets; the pieces are filled up through the conical tube. When emptied, the pieces can be removed to the cask-washing department and treated there. This system is carried out in some of the older London breweries as follows: The wort after being attenuated to a certain degree in the square or round, is run off into a series of barrel-like vessels called *pontos*, which hold from four to six barrels, in which the fermentation is completed. While the fermentation is in progress, the yeast forces itself out through a lipped opening in the head of the *ponto*, and flows into a slate gutter below, lying between rows of *pontos*. As the yeast works its way out, the *pontos* are kept filled with fermenting beer, by a "feed-back" situated above them.¹ This system is now generally replaced by the dropping system (see p. 537).

Burton Unions.—These are considered by many to be the highest development of the cleansing system. In this system, as represented in Fig. 94, the casks, which are of about four barrels' capacity, are permanently mounted on tall wooden stands, to which they are slung by means of two axles, one attached to each head. These work in bearings, and permit the cask to be rotated on its axis, for which purpose one of the heads of the trunnions is made square, so that a handle may be attached to it when necessary. The bung-hole of each cask is provided with a conical brass socket, into which fits a

FIG. 94.—Burton Union.

hollow brass plug, carrying a pipe to convey away the yeast. This is called the "swan-neck," and consists of a tinned copper tube, which, after being carried up vertically a foot and a half or two feet, makes a turn of half a circle, curving over into a long wooden trough, which extends between the two rows of adjacent casks, and is called the "yeast trough." At one end of this another vessel is fixed, called the "feed trough," which has a capacity of five or six barrels. A tap is fixed into the bottom of this, from which a pipe of about two inches in diameter proceeds, extending in front of each row of unions, and giving off a short branch to each cask, with which it can be connected by means of a union joint to a tap permanently fixed in the head of the cask. Another cock is fixed in each cask exactly opposite the bung-hole, and is provided with a short tube which projects some little distance inside the cask, and which can be raised or lowered by means of a screw. This serves for the removal of the fermented beer, and, as the tube communicating with the tap is some little height above the bottom, it serves to hold back the bottoms. When a set of unions are cleansed, the swan-necks are first removed and the feed-pipe communications unscrewed; the handle

¹ J. L. Baker, *The Brewing Industry*, Methuen & Co.

is then attached to each cask in turn, boiling water poured into it, and the cask rotated on its axis; this is an objectionable feature in the system, for the introduction of large quantities of hot water into the fermenting-room necessarily raises its temperature.

Attemperators.—These are of two varieties, fixed and loose. The former consists of a coil of tinned copper which runs round the inside of the fermenting-vat, to the sides of which it is fixed by gun-metal brackets, which support it at such a distance from the sides of the vat as to afford sufficient room for cleaning purposes. The first round of the coil runs just under the surface of the wort; each succeeding turn is about six or eight inches lower than the preceding one, and, being supported on a longer bracket, stands out an inch or two farther into the tun. Cold water enters through the regulating tap at the upper extremity of the coil. The other extremity turns upwards on its exit from the vat, and is carried up to a level a few inches higher than the uppermost round of the coil, so that the whole of the attemperator is kept completely filled when in action. The end of the pipe then turns over into the funnel communicating with the waste-pipe, and this portion of the apparatus is placed in such a position that the person regulating the supply can readily see the quantity of water running away.

The movable attemperator is a flat coil of pipe, either fixed in a wooden frame, or, what is better, held together by metal connections. It is suspended by chains, by means of which it can be lowered or raised. The communication with the water-supply and waste-pipe are made with india-rubber tubes. Sometimes this kind of attemperator, especially when it is to be used in long, narrow tuns, takes the form of a gridiron, the pipe being bent backwards and forwards. In any case, the apparatus should be easily removable from its attachments, so that it can be taken down and thoroughly cleaned. According to Southby, fifty-five square inches of cooling surface are required to each barrel of wort when working on the skimming system. This will be, where a one-inch pipe is employed, one and a half feet, or one foot where the pipe is one and a half inches in diameter.

Arrangements for the Removal of Yeast.—Small squares or rounds are often skimmed by hand with an appliance something like that used for skimming milk, but when the fermenting vessel is of any size the operation becomes tedious and troublesome, and some form of mechanical skimming is necessary. In rounds, the removal of the yeast is effected by one of the two forms of “parachute” and skimming-board. The first of these consists of a funnel attached to a pipe which passes through a packed gland fixed in the bottom of the tun. By an arrangement of rack-and-pinion work the mouth of the parachute can be moved upwards or downwards; it is placed about half an inch higher than the level of the wort. A valve is fixed in the throat of the funnel, to which a chain is attached. The parachute is placed in the centre of the round, and the skimming-board, which in this case is a strip of sheet-metal about six inches broad, takes the form of a helix mounted on an axle situated in the axis of the tun. The skimming-board, like the parachute itself,

is provided with an arrangement by which it can be raised or lowered, and also another for rotating it. The board is placed in such a position that its lower edge just touches the surface of the wort, and in the course of a revolution in one direction it brings the yeast from the sides into the centre; the yeast then falls into the mouth of the parachute. In the second and more usual form, illustrated in Fig. 95, the funnel has the shape of a small sector of a circle, and extends from the centre to the circumference of the tun, the pipe

FIG. 95.—Fermenting Round with Parachute.

from it having an eccentric position. The skimming-board in this case is straight, a little shorter than the radius of the tun, and is attached by one of its extremities to an axle rotating in the axis of the tun. In a single sweep round it pushes the yeast before it into the mouth of the parachute. Arrangements for raising and lowering the parachute and skimming-board are provided; also a valve in the throat of the former. The parachute arrangement is sometimes applied to squares. In this case the funnel has the shape of a long thin parallelogram, situated at one end of the vessel. The skimming-board is straight, and moves on a travelling carriage, and in its passage from the end of the tun farthest from the parachute pushes the yeast before it into this apparatus. Squares are sometimes provided with a yeast-slucie, which consists of a flat plate of wood or metal having a projecting lip. This is fixed against

an opening cut out at the top of one of the sides of the square, arrangements being provided by which it can be raised and lowered. A strip of india-rubber between the sluice-board and the side of the square renders the whole water-tight.

Rousers and Aërotors.—The yeast is mixed into the wort by hand-rousters, each of which consists of a flat board with a hole in its centre by means of which it is attached to the end of a long wooden handle. A simple contrivance for rousing and aërating at the same time consists of a small cask of about three gallons capacity, with both its ends removed, and having a number of holes bored through its sides. It is weighted with lead so as to sink readily, and suspended by a rope passing over a pulley. The cask is let down into the fermenting wort and pulled up suddenly to a short distance above its surface; by repeating this several times, a very efficient rousing and aëration are secured.

There are numerous forms of rousing apparatus worked by power, such as an arrangement similar to the screw propeller of a ship, a current of air forced in by a pump or bellows, or a centrifugal pump, which draws the wort from the bottom of the tun and delivers it at some height above the surface of the wort. The extremity of the delivery pipe may be made to terminate in a rose with fairly large holes; this divides the wort into a number of small streams, and secures an abundant aëration. By causing the pump to turn in the contrary direction, air may be forced into the wort.

Racking Squares.—Beers brewed on the skimming or cleansing system are often not racked off directly from the fermenting vessels, but run into a vessel which may be large enough to hold two brewings. These may be made of wood or of slate, the latter being much the better material for the purpose. They should not be above four feet deep, and should be fixed at such a height that the largest casks can be filled from them conveniently. A number of taps are inserted in the front of the racking square a few inches above the bottom; and to these, lengths of india-rubber hose are attached long enough to reach to the bottoms of the casks to be filled. To the end of each piece of hose a racking tap is often attached, the nozzle of which has a length of metal tube attached, sufficiently long to reach the bottom of the cask. When a number of small casks are being filled, a certain amount of succession takes place in the hose each time the racking tap is closed, which being transmitted to the beer in the tank, disturbs the yeast. This may be avoided by placing an intermediary vessel between the racking tank and the hose, the wort flowing into this vessel from the tank through a ball-cock.

Vats and Casks.—The large vats for storing purposes are made of the best English oak, hooped with iron. Since they are subject to the attacks of a fungus which causes dry rot, they should be examined periodically, and measures for the eradication of the fungus taken as soon as its presence is detected.

The trade casks are generally made of foreign oak, occasionally of English. The best quality of foreign oak comes from Memel, the next best from

Dantzic, other kinds from Odessa, Blumeza, and Riga. Ale casks have been constructed from time immemorial of wood, which is a substance of a porous nature, into the interstices of which bacterial and other minute organisms are extremely liable to effect an entrance. When certain species of these organisms have, in this way, invaded the substance of the wood, it is next to impossible to effect their removal. A cask so attacked is known as a "stinker," in which condition it is completely useless for the carriage of beer. On the Continent the casks are coated internally with a peculiar kind of pitch, so that the beer never comes into actual contact with the wood. Probably in the future some material which does not take up impurities so readily as wood will be employed in the construction of brewery casks, such as steel lined with tin, or wood with a lining of some indifferent metal.

CHAPTER IX.

ESTIMATION OF THE QUANTITIES OF MATERIALS FOR THE BREW.

BEFORE commencing the operation of brewing, it is necessary to calculate, or, in other words, work out the quantity of materials which will give the required quantity of beer of the desired gravity. This is a very simple matter, and it is here that the great convenience of the expression "brewers' pounds of extract" is seen. All that has to be done is to reckon up the total number of pounds of brewers' extract which will be contained in the number of barrels of beer, or, as it is generally called "in the length of the brew," and then to reckon up the quantity of malt, or of malt and malt-substitutes, which will be required to furnish this amount of extract.

Suppose, for example, we wish to brew 150 barrels of beer at a specific gravity of 1050. Converting this into brewers' pounds ($1050 - 1000 \times 0.36 = 18$), we find that 18 lb. per barrel corresponds to a specific gravity of 1050. The number of barrels in the length to be produced is then multiplied by the number of pounds of extract ($150 \times 18 = 2700$); thus we find the total number of pounds required will be 2700. If the brew is to be entirely from malt, the actual yield of extract per quarter of which we do not know, we take the average yield of malt, which let us assume is about 86 lb. per quarter. Dividing the 2700 by 86, we obtain 31.395 quarters, and $0.395 \times 8 = 3.16$, or a trifle over three bushels. The quantity to be entered in the Excise-book will be therefore 31 quarters 3 bushels, if the malt weighs 42 lb. to the bushel. If it does not, then its weight must be calculated into bushels of 42 lb. weight. Suppose the malt only weighs 40 lb., then, as $42 : 40 :: 31.395 : 29.9$, or it will be entered as 29 quarters 7 bushels. If a portion of the extract is to be derived from sugar, then this amount must be deducted from the total amount required for the brew. Suppose, in the foregoing brew, one-fifth of the extract is to be obtained from sugar, then, as $2700 \div 5 = 540$ lb. of extract is to be derived from sugar, consequently $2700 - 540 = 2160$ will have to come from malt—that is, $2160 \div 86 = 25.12$, or 25 quarters $9\frac{1}{2}$ bushels will be required. If the sugar gives 35 lb. of extract per cwt., then $540 \div 35 = 15.42$ cwts. This figure, multiplied by 112, gives 1727 lb. (which is the quantity to be entered in the Excise-book), or 15 cwts. 47 lb.

When several beers of different gravities have to be produced from one mashing, or, in other words, the wort to be divided into "party gyles," the

calculation is made in a similar manner. Supposing that three different beers have to be produced with one mash, say 80 barrels at 24 lb., 60 at 20 lb., and 50 at 14 lb., then the total extract required will be $80 \times 24 + 60 \times 20 + 50 \times 14 = 1920 + 1200 + 700 = 3820$. From this amount the malt, or malt and sugar, required is calculated as before. When gelatinised materials, such as flaked rice or maize, are to be employed, since these yield extracts of from 100 to 105 lb. per quarter of 336 lb., a corresponding allowance must be made. Thus, suppose 520 lb. of the extract of the brew are to be obtained from flaked maize, yielding an extract of 104 lb. per quarter, $520 \div 104 = 5$ quarters (1680 lb.) will be required. This, under the present regulations, has to be entered in the Excise-book in the proper column for prepared grain. As, however, maize is bought by the cwt., and also used in the brewery by weight, the 5 quarters will have to be calculated into cwts., and as there are 3 cwts. to the quarter, the number is simply multiplied by 3.

Calculation of the Amount of Liquor required for the Brew.—

As there are several sources of loss of liquor on its way from the hot-liquor back to its ultimate destination, the fermenting vat, much more liquor is needed for a brew than the actual quantity, or the length, of the wort which is to be collected. For instance, we commence in the mash-tun with dry malt and leave off with wet grains; these absorb on an average from 28 to 30 gallons of liquor for every quarter of malt used. In the subsequent boiling which ensues in the copper, a further quantity of liquor is lost by evaporation, and this differs considerably, according to the shape of the copper, the vigour of the ebullition, the state of humidity of the atmosphere, &c.; as an average 8 per cent. per hour may be set down for this. The wort, when on the cooler also loses water by evaporation, and to a slight extent also in its passage over the refrigerator; these two sources of loss may be taken collectively at about another 8 per cent. Some little, about 2 per cent., remains adherent to the various vessels it passes through. Finally, there is the liquor retained by the hops, which may be set down at about $1\frac{3}{4}$ barrels for each 100 lb. of hops, or a little over half a gallon for each pound. The total amount of liquor lost by evaporation from copper, cooler, and refrigerator, and adhering to vessels proportionate to the length of a brew, after being once ascertained, will be found to be tolerably constant for each brewery; as a rule, it averages about 30 per cent. The other two, the liquor absorbed by the malt and by the hops, will naturally vary with the respective quantities of each employed in different brews. Taking, as an example, a brewing in which 75 barrels of wort, of the gravity of 17 lb. (sp. gr., 1047), are to be collected in the fermenting vessels, and in which hops are to be used at the rate of 8 lb. per quarter of malt, taking malt which yields 85 lb. extract per quarter, the number of quarters required will be 15, and $15 \times 8 = 120$, the number of pounds of hops required. The 15 quarters of malt will absorb $15 \times 30 = 450$ gallons, or $12\frac{1}{2}$ barrels of liquor, and the hops will retain $120 \times 0.0175 = 2.10$ barrels nearly. The loss from these two sources amounts, therefore, to 14.6 barrels. Beyond this,

there is the further loss of say 30 per cent. on the 75 barrels of wort required, which is found by multiplying 75 by 0.30, and gives $22\frac{1}{2}$ barrels. This, with the 14.6 mentioned before = 37.1 barrels, and with another 6 barrels for contingencies, together with the 75 barrels of finished wort, will give a total of 118 barrels.

Heating the Mash Liquor.—In all properly arranged breweries, a hot-liquor back is provided in which water for the mash is heated by steam. Although, theoretically, it is not necessary to raise the temperature of the mash liquor beyond some 25° or 30° above that at which it is actually required to enter the mashing-machine, it has been found by practical experience always best to boil the liquor for a quarter or half an hour. The contents of the hot-liquor back are heated by steam, which may either pass through a coil, or, in breweries where the steam is perfectly free from contamination, it may be blown directly into the water; or, as it is termed, the liquor may be heated by “naked steam.” The boiling of the mash liquor generally takes place on the evening before brewing, and during the night it will have cooled down to a lower temperature than that required for mashing; steam is therefore turned on for a short time in order to bring the liquor up to the proper temperature. In the boiling which the mash liquor is subjected to, obviously all living organisms and their germs will have been destroyed. Since the majority of waters contain acid calcium carbonate in solution, and this, by boiling, is decomposed into insoluble calcium carbonate (chalk), with liberation of carbon dioxide, a precipitate forms which has the effect of carrying down with it a large portion of the organic matter of the water. It is extremely undesirable that this precipitated calcium carbonate should obtain access to the mash-tun, because it would there partially neutralise the natural acidity of the malt; consequently, the aperture from which the mash liquor is drawn off should not be situated at the bottom of the back, but at its side some few inches above the bottom.

Addition of Hardening Materials.—If calcium sulphate or other salts are to be added to the mash liquor, this is best done when it is on the boil. The gypsum and other substances are mixed with water to the consistency of thin whitewash, and poured into the hot-liquor back when its contents are in a state of lively ebullition.

Many breweries are unprovided with a hot-liquor back, and in these the liquor is heated either in a separate copper or in the brewing copper itself. In such cases the heating and treatment of the liquor must be made to approximate to the method just described as nearly as the nature of the plant will permit.

Mashing.—This operation consists in intimately mixing the ground malt or “grist,” as it is termed, with a definite proportion of water at the proper temperature. The mixture must be thorough, for any portions of malt which escape wetting are withdrawn from the action which ensues on mashing, and as their contents remain unaffected, they represent so much waste material. This can hardly apply nowadays to places where mashing-machines are erected,

for, unless these are worked in an extremely careless manner, they do their work most efficiently.

Temperature of the Mash.—From what has gone before it will be seen that the temperature at which the mash is conducted has an immense influence on the nature of those changes which the starch undergoes under the action of the diastase, and these, as will be seen afterwards, exercise a profound influence on the subsequent fermentation and on the character of the finished beer. The most important temperature is that known as the “initial temperature” of the mash, for upon it chiefly depends what the nature of the products formed by the action of the diastase upon the starch constituents of the malt shall be. The relation between the temperature of the mash liquor (“striking heat”) and the initial temperature of the mash has been discussed in a previous portion of this work. On p. 514 the useful table compiled by Brown and Morris (compare p. 11) is given. This temperature, which is taken as soon as the mashing process has been completed, is ascertained by means of a thermometer constructed especially for this purpose. The initial temperature is varied according to the quality of the malt and the character of the required beer. Beers intended for storage are, as a rule, brewed from pale malts. These are mashed at a somewhat higher degree of temperature than high-dried malts, for we require, in this class of beers, a fairly large quantity of those dextrins which ferment very slowly, and which, since they provide for a long, slow, continued fermentation, keep the ale (through the period of its storage) charged with gas. High-dried malts are mashed at a somewhat lower temperature, since the beers produced from them are quickly consumed, and here we require a wort which contains large quantities of maltose and of those maltodextrins which give fullness and sweetness to the beer. Between these two types of beer, which may be taken as extremes, there are many other intermediate ones that are brewed to suit the particular wants of different neighbourhoods. The mashing temperature will also vary according to the diastatic power of the malt. Malts in which this is high will require a higher mashing temperature than less diastatic ones. There is, excepting in very rare cases, an excess of diastatic power in all brewing malts, and this has to be checked or crippled to a certain extent in the mash-tun; hence heats have almost invariably to be employed, which destroy a certain portion of the diastase. If such were not the case, too much maltose would be yielded, and the resulting beer be deficient in fullness and body. The temperatures for the initial heat vary from about 145° F. to 155° F. Malt which is perfectly dry will require a somewhat lower mashing heat than malt which has become to a certain extent slack, because, when perfectly dry starch is mixed with water, a certain amount of heat is evolved owing to the chemical combination which takes place between the two. This slightly increases the heat of the mash, and naturally the elevation of temperature from this cause is greater the drier the malt. A delicate appreciation of the right initial heat to be employed with different malts to produce beers of the required character is only to be obtained by actual experience in working, and is one of those things for which hard and fast rules cannot be laid down.

TEMPERATURE OF MASH.

Table showing the Temperatures in degrees F. resulting from mixing Malt and Water of different Temperatures when quantities and Specific Heats only are taken into consideration.

Specific heat of Malt containing 2 per cent. of water = 0.53 (dry malt = 0.52).

Water temp.	Malt at 55° F. Barrels per quarter.				Malt at 60° F. Barrels per quarter.				Malt at 65° F. Barrels per quarter.				Malt at 70° F. Barrels per quarter.			
	2	2½	2¾	2	2	2¼	2½	2¾	2	2¼	2½	2¾	2	2¼	2½	2¾
170	147.1	149.3	150.9	152.3	148.2	150.2	151.8	153.0	149.2	151.2	152.6	153.8	150.1	152.0	153.4	154.6
169	146.3	148.4	150.1	151.4	147.4	149.3	150.9	152.2	148.4	150.2	151.8	153.0	149.3	151.1	152.6	153.7
168	145.5	147.6	149.3	150.6	146.6	148.5	150.1	151.4	147.6	149.4	150.9	152.1	148.5	150.3	151.8	152.9
167	144.7	146.8	148.5	149.8	145.8	147.7	149.3	150.5	146.8	148.6	150.1	151.3	147.7	149.5	150.9	152.0
166	143.9	146.0	147.6	148.9	144.9	146.9	148.5	149.7	146.0	147.8	149.2	150.5	146.9	148.7	150.1	151.2
165	143.1	145.2	146.8	148.1	144.2	146.1	147.6	148.8	145.2	147.0	148.4	149.6	146.1	147.9	149.3	150.3
164	142.3	144.3	146.0	147.2	143.4	145.2	146.8	148.0	144.4	146.1	147.6	148.8	145.3	147.0	148.5	149.5
163	141.5	143.5	145.1	146.4	142.6	144.4	146.0	147.2	143.6	145.3	146.7	147.9	144.5	146.2	147.6	148.6
162	140.7	142.7	144.3	145.6	141.8	143.6	145.1	146.3	142.8	144.5	145.9	147.1	143.7	145.4	146.8	147.8
161	139.9	141.9	143.5	144.7	141.0	142.8	144.3	145.5	142.0	143.7	145.1	146.3	142.9	144.6	146.0	146.9
160	139.1	141.1	142.6	143.9	140.2	142.0	143.5	144.6	141.2	142.9	144.2	145.4	142.1	143.8	145.1	146.1
159	138.3	140.2	141.8	143.0	139.4	141.1	142.6	143.8	139.4	142.0	143.4	144.6	141.3	142.9	144.3	145.2
158	137.5	139.4	141.0	142.2	138.6	140.3	141.8	143.0	138.6	141.2	142.6	143.7	140.5	142.1	143.5	144.4
157	136.8	138.6	140.2	141.4	137.8	139.5	140.9	142.1	137.9	140.4	141.7	142.9	139.7	141.3	142.6	143.5
156	135.9	137.8	139.3	140.5	137.0	138.7	140.1	141.3	137.0	139.6	140.9	142.1	138.9	140.5	141.8	142.7
155	135.1	137.0	138.5	139.7	136.2	137.9	139.3	140.4	136.2	138.8	140.1	141.2	138.1	139.7	141.0	141.8
154	134.3	136.1	137.7	138.8	135.4	137.0	138.4	139.6	135.4	137.9	139.2	140.4	137.3	138.8	140.2	141.0

Note 1.—The above numbers for the heat of mixing malt and water at the specified temperatures will be reduced by 0.2° for each percentage of water in the malt above 2 per cent. Thus, when malt at 60°, containing 4 per cent. of water, is mixed with 2 barrels per quarter of water at 170°, the resulting temperature will be 147.8°, and not 148.2°.

Note 2.—For temperatures of malt intermediate between those given above, add to the mixing temperatures:

When mashing with 2½ barrels per quarter 0.16°
 " " " " " 0.14°
 " " " " " 2¾

When mashing with 2¼ " " 0.18°

for each degree above 55°, 60°, 65°, and 70° respectively.

The Operation of Mashing.—Before the actual mashing is commenced the mash-tun is thoroughly warmed, either by being partly filled with hot water which is afterwards run to waste, or it is heated by steam. In the actual mashing, where the tun is provided with a mashing-machine, water is run through this from the hot-liquor back, the contents of which have been brought to the proper mashing temperature, or, as it is often termed, the right “striking heat,” until the plates are just covered. The slide of the grist-case is then opened, and the malt allowed to fall into the mashing-machine, where it is intimately mixed with the mash liquor. The consistency of the mixed mash and water as it falls into the tun is observed, and by a little practice the beginner is soon able to appreciate the stiffness of the mash which corresponds to a definite proportion of water to malt, and to regulate the flow of liquor and grist accordingly. The proportions generally used are from one and a half to two and a half barrels of water, or “mash-liquor,” as it is generally termed, to each quarter of malt. The former would be considered a very stiff mash, and the latter a thin one. Obviously, the less liquor employed in the mash the more there remains for sparging, and the better chance there is for obtaining all the extract from the malt in the tun, or, as it is now termed, the “goods.” Diastase exhibits a little more activity in a thin mash than in a stiff one. Mashing when performed in this way is carried out in the most perfect manner, for each portion of grist meets its own portion of water in the mashing-machine, and an absolutely uniform heating of the entire mash is secured.

In the case of the mash-tun which is not provided with a mashing-machine but with rakes, rather more than the total quantity of liquor required for the mash is run into the tun at a temperature a few degrees higher than that actually to be employed in the mashing. The object of this is to provide a sufficiency of heat to warm the mash-tun; the extra quantity necessary for this purpose will have to be determined by experiment for each particular tun. After the tun is sufficiently heated the taps are allowed to run for a few minutes so as to flush out the spend-pipes; they are then closed and the temperature of the mash liquor taken. This should now be a degree or two above the temperature actually required; a few revolutions of the rake machinery will quickly reduce it to the proper temperature. When this is reached the mash-tun is closed, the slide in the grist-case opened, and the grist allowed to fall rapidly into the mash liquor, the rakes being kept in motion at the rate of about one revolution round the tun in forty or fifty seconds. The rakes are kept on for about fifteen minutes after all the malt is in the tun, and then the temperature of the mash is taken.

In certain breweries the malt is run into the tun first and the hot liquor afterwards; this is an exceedingly faulty method, and is only mentioned to be condemned.

The latter two methods of mashing have this great disadvantage, that by neither of them is it possible to ensure that each portion of grist is raised to the same temperature in the mashing. Obviously, those portions which come

in contact with the hot liquor first are raised to a much higher temperature than the last portions; consequently, the diastatic power of the former is considerably crippled, whilst that of the latter portions is scarcely, if at all, affected. Such a mash is therefore of a very irregular nature in this respect.

Some brewers who have no mashing-machine endeavour to get over this defect by running in the hot liquor and grist at the same time, keeping the rakes continuously in motion; this, no doubt, obviates to some extent the imperfection, yet it can never yield such a regular mash with regard to equable temperature as the mashing-machine affords.

Underlet.—It is customary after the mash has stood on for some little time, a quarter to half an hour, to admit a further portion of hot liquor, at a higher temperature than that used in mashing, under the goods by means of the underlet. Such an addition also goes by the name of “piece” liquor. The temperature of this varies with the temperature of the liquor employed in mashing, and is generally about 10° to 12° above it. In those cases where the mash-tun is unprovided with rakes, the piece liquor should be allowed to flow in slowly, so as to secure as even an admixture as possible under the circumstances. Where the mash-tun is provided with rakes, after the piece liquor has been added, they are slowly revolved three or four times, in order to secure an even temperature throughout the goods.

Standing on.—The mash is then allowed to stand in the mash-tun until the conversion of the starch is completed. This is ascertained by taking out from time to time a small quantity of the liquid portion of the mash and testing it with iodine. In order to do this, a few drops of the mash are placed on a white tile or the bottom of an ordinary dinner-plate,¹ allowed to become cold, and a drop of a solution of iodine potassium iodide, about the colour of brown brandy, added. If this experiment is tried as soon as the mash is made, a deep blue colour will be produced. As the mashing process proceeds the colour gradually changes from purple to brownish-red, and at last no colour is yielded at all, the wort being simply coloured yellow by the iodine solution. As soon as this happens the taps may be opened and the wort run off.

Second Mash.—In some breweries, where the copper is not large enough to boil the whole mash at once, the mash, after being made in the usual manner, is sparged until one-half of the total length required is obtained. This is run or pumped up into the copper, and fresh mash liquor run on to the goods at the rate of about one and a half barrels per quarter, and allowed to stand on until the first half of the length in the copper has been sufficiently boiled. When this has been run off the taps are again set, and sparging carried on until the second half of the length is reached, which in its turn is brought into the copper and boiled. Very occasionally a third mashing is made in a similar manner. The second mash is a time-wasting arrangement, though it is strongly advocated by some brewers, who consider that it gives a better yield of extract. Opinions are, however, somewhat divided as to the

¹ Small slabs of plaster of Paris are also used for this purpose.

value of the last portions of extract obtained in this way; some maintain that it is not only useless, but even prejudicial.

Use of Subsidiary Apparatus.—A number of these have been described, such as circulators, steam-coil in the mash-tun, &c., the object of all of them being to raise the temperature of the mash gradually. This may be performed for two very different purposes: either to increase the quantity of the less fermentable maltodextrins, or to deal with inferior or badly germinated malt.

In those cases where the circulator is used for raising the percentage of the higher maltodextrins, the apparatus is set in action ten minutes after the mashing is completed, and the operation continued for fifteen minutes, or for such longer period as is required to bring the mash up to the required temperature. The mash may be raised in this way to 158° F., or even higher, if it is necessary to restrict the diastase considerably. In this case it is usual to curtail the time of standing on to one hour, reckoning from the time the circulation is finished. One advantage of the use of a circulator is that the wort flows bright in a much shorter time after setting taps than it does under the ordinary conditions.

The same object may be effected by the use of a steam-coil under the plates as first suggested by Dr. Charles Graham. The mash is made with a low initial temperature, 130° to 134° F., and directly this is completed, steam is turned on at such a rate as will bring the temperature of the mash up to 160° F., or even to a few degrees higher, in from thirty to forty minutes. A stir round with the rakes is given every seven or eight minutes to distribute the heat. It must not be forgotten that under these conditions a very high temperature is required to cripple the diastase, since it is protected by the maltose, which is, in these circumstances, rapidly formed. The time of standing on may, with advantage, be considerably shortened when this method is employed.

When using any of these arrangements for dealing with badly vegetated malts, which are always more or less deficient in diastatic power, the mash should be made at a low initial temperature, such as 140° to 145° F., and, after remaining at this temperature for half an hour or so, the heat should be very slowly and gradually raised to 155° F., at which temperature the mash should be allowed to stand until it ceases to give a coloration with iodine. The object of this treatment is to dissolve out as much diastase as possible, and then assist its action by the subsequent heating up of the mash. Circulating, unless very high heats are employed, promotes diastatic action.

Hot Grist Mashing.—In this modification of the mashing process, which was introduced and patented by Mr. Charles Clinch, the grist is heated by means of hot air to a temperature slightly lower than that of the initial mash heat required, and the mash liquor is brought to the same temperature. A slight rise in temperature takes place in the mashing, owing to the heat developed by the combination of the starch with water, and this brings the mash up to the correct initial. The malt is said to be benefited by this heating process, its aroma being brought out. During the standing on, diastatic action proceeds with extreme vigour, and the mash must not be allowed to stand

more than an hour. Brilliant dextrinous worts, which contain an abundance of food for the yeast, are said to be obtained by this method.

Limited Decoction.—This process seeks to combine, to a certain extent, the decoction processes employed on the Continent in the production of lager beer with our infusion system. A mash-tun provided with a steam-coil is employed. The mash is made in the usual manner with about two barrels to the quarter, at an initial temperature of 155° F. or a little higher. After standing for an hour, taps are set and wort run off at the rate of half a barrel per quarter, and this is kept in a suitable vessel until required. Steam is then turned on and the goods raised to a temperature closely approaching 212° F., at which it is kept for fifteen minutes. Cold liquor is now sparged on until the mash is reduced in temperature to about 160° F., with the rakes going all the time. The strong wort which was drawn off previously is now stirred in, the temperature of the mash brought up to 160°, if necessary. The rakes are then stopped and the mash allowed to stand for twenty minutes or half an hour, after which the taps are set and the remainder of the operation performed in the usual manner. A little more extract is obtained in this way from good malts, but the benefit is most marked when working with hard, steely, or ill-vegetated samples. Unless the plates are good and the perforations fine, there may be trouble with the drainage.

As in this method the starch is completely gelatinised, it is possible to use a portion, say 25 per cent., of raw grain.

American System of Using Large Amounts of Raw Grain.—The American brewers use as much as 50 per cent. of maize in the shape of grits, which are thoroughly gelatinised in a separate vessel, and cooled down to the mashing temperature. The mash is made with the malt in the usual manner, and after being allowed to stand on for from an hour to an hour and a half, the gelatinised maize is stirred in, and the whole allowed to stand on for a very short time, when the taps are set. As the starch of the maize is thoroughly gelatinised the diastase acts upon it with extreme rapidity. When such large percentages of raw material as these are employed, a distinct kind of malt is necessary, made from the coarse-skinned varieties of barley, in order to facilitate the drainage in the mash-tun. The barley is fully germinated during the malting process, and the resulting malt dried at a low temperature, so that it possesses a maximum diastatic power.

This plan of using maize grits has been adopted to a limited extent in this country, and vessels called “converters” have been placed on the market. The maize grits (which should not contain more than one per cent. of oil) are introduced into the converter together with about five per cent. of ground malt; steam is then turned on, and the vessel and its contents brought up to 200° F., and kept at this temperature for half an hour. The gelatinised maize which is partly converted into soluble starch, is mixed with the goods in the mash-tun just before the taps are set.

Employment of Prepared Materials.—Within the last few years a number of materials, which are more or less gelatinised, have been introduced as partial

substitutes for malt. One of the first of these was a grist in the form of a coarse powder, in which state it was apt to give rise to drainage troubles. Subsequently rice was gelatinised and rolled into flakes; and soon after this maize, deprived of its germ which contains a large quantity of oil, was similarly treated. Barley is flaked in a similar manner. Barley and maize are also torrefied by being passed through a hot revolving cylinder; the sudden application of heat ruptures the starch-cells and bursts the corn, the contents of which are left in a tender friable condition, and can be readily disintegrated by the malt-mill.

In using these materials no alteration is made in the method of mashing; they are simply mixed with the malt, and pass along with it through the malt-mill, or, in the case of the flaked preparations, they may be mixed with the grist immediately after it is passed through the mill. As a rule from 10 to 20 per cent. of this class of material is employed.

From practical experience one of the authors (A. R. Ling) is unable to recommend unqualifyingly the use of any form of raw grain, prepared or otherwise, as these invariably contain, besides starch, a greater or smaller amount of substances which tend to impair the flavour and stability of the beer. Among these may be mentioned albuminoid bodies which cause turbidity and instability, pentosans which may give rise to thinness and bad flavour, and oil, which, besides imparting an objectionable flavour, exercises a prejudicial effect on the head-retaining properties of the beer. Raw grain cannot be regarded as a malt substitute in the same sense as sugars, the service of which is generally admitted. In the case of the very best beers, any form of raw grain is best omitted altogether, and the authors are inclined to place the limit at which these materials may be used for any description of beer without prejudicing its quality at 10 per cent. of the total grist calculated on the extract yielded.

Black Beers.—In the production of stout and porter it was formerly the custom to use a grist composed of pale, brown, and black or patent malts. Sometimes the brown malt is omitted, but this is attended by loss of flavour and fulness; often a portion of the black malt is replaced by caramel. The relative proportions of these constituents vary considerably in different breweries, as is shown in the following table:—

	Pale.	Brown.	Amber.	Black.
	Per cent.	Per cent.	Per cent.	Per cent.
No. 1	48	24	24	4
No. 2	57	25	15	3
No. 3	58	30	10	2
No. 4	60	34	0	6
No. 5	91	0	0	9

It is also customary to use a certain amount of sugar in black beers; this is generally either invert or cane.

Return Wort.—In some breweries it is the rule to pump the last runnings from the mash-tun, or the liquor which has been used to sparge the hops, or both, into the hot-liquor back, and to use them for mashing in the next day's brew. This plan is of very doubtful value, for though the last runnings may contain a certain amount of extract, as shown by the hydrometer, yet it is extremely questionable if it is of much value. When hopped returned worts are used, they have a great tendency to cause what is known as a "dead" mash. Probably this is owing to the precipitation of the proteids of the malt by the tannin bodies of the hops; the diastase is also prejudiced from the same cause.

Setting Taps.—This is the designation given to the opening of the taps which are attached to the ends of the spend-pipes. The taps are first turned on very slightly, and as soon as the wort begins to run clear they are then gradually opened more fully. After the sparging is commenced they are adjusted so that the quantity of wort flowing from them is about equal to the amount of liquor being delivered by the sparge, but, as will be seen farther on, this method is subject to modifications.

Sparging.—In order to obtain the full amount of extract from the goods in the mash-tun, it is necessary that the sparging should be performed in an intelligent manner, and that the apparatus should be well arranged and in good order. Sparging should never be too rapid; it should, as a rule, occupy about four hours. Very often spargers are badly constructed, and do not deliver the liquor evenly on the surface of the goods; and the same thing arises if a number of the holes in the arms of the sparger become blocked up with dirt. In order to ascertain if a sparger is working evenly, it should be tested with the trough described on page 490.

With reference to the heat of the sparge liquor, this should never be too high, or during the last part of the operation the diastase present in the mash may be completely destroyed, and soluble starch may make its appearance in the wort and give rise to subsequent difficulties. Since, during the time of standing on, the goods at the surface lose a certain amount of heat, it is customary to run on the first portions of the sparge liquor at a somewhat higher temperature than the latter portions. As a rule, the first half or three-quarter barrel of sparge liquor per quarter may be run on at a temperature of from 170° to 175° F. The temperature should then be allowed to decline gradually, until, at the final stage of the process, the temperature stands at 160°, or a few degrees lower.

Much difference exists as to the way the sparging is performed in relation to that in which the wort is drawn off. Some brewers sparge at the same speed as that at which the wort is running off, in which case the goods are kept at about the same level all through the operation. Others, after stopping the sparge, allow almost the whole of the wort to run off once or several times during the process. The former is probably the better plan for beers of low gravity, the latter for stronger ones, and this latter course becomes a necessity when a large quantity of concentrated wort is required. In this last case as

much wort as possible is run off, the amount of wort drawn off being replaced by sparge liquor, or by underlet, or by both, two or three revolutions of the rakes given, and the whole allowed to stand for a short time before sparging is again commenced.

This plan of adding liquor by underlet, mixing with the rakes, and allowing a short stand, may be resorted to if the drainage becomes bad at any time from the goods getting down on the plates. Care should be taken to see that the sparge liquor does not form channels through the goods, a contingency which may occur, especially at the sides of the rake machinery. All the liquor passing through such channels escapes without contributing its share to the washing-out process.

The last runnings, if the sparging has been carefully and properly performed, should have a gravity of not more than 1004 sp.gr. for worts of medium strength, and a couple of degrees higher than this for strong worts; and this is perhaps as far as the washing should be carried, for, according to some authorities, if carried farther, substances of doubtful value are extracted. A quantity of liquid nearly equal in weight to the original weight of the malt used in mashing remains in the grains. (Compare, however, pp. 492-496.)

Dead or Set Mash.—During the whole sparging process the goods should float on a substratum of wort, and their surface appear comparatively dry. When from any cause the goods fail to float in this way, they are said to “set” and the mash is described as “dead.” In such cases, the goods lie obstinately on the plates, the sparging liquor, instead of percolating through them, collects on their surface, and flooding ensues. This state of affairs may arise from several causes: too high a striking heat may have destroyed the diastase; the malt may have been too finely ground; malt-flour may have been separated from the husk by falling from too great a height into the grist-case, or by excessive knocking about in the elevator; the employment of too much huskless material; and excessive use of the rakes. Sometimes, when bad drainage depends upon a layer of starch becoming deposited on the plates, this after a time is dissolved by the action of the diastase, and the drainage then improves. Occasionally, a stir round once or twice with the rakes will mend matters, but in very bad cases such methods as draining off from the grain trap, or placing a bundle of straw in the goods and pumping from the middle of this have had to be resorted to. A dead mash is, however only the result of carelessness. (Compare p. 495.)

Wort in Underback.—In some breweries this vessel is a necessity, because the copper is at a higher level than the taps, and the wort must have some receptacle to lodge in until it can be pumped into the copper; in others, where the mash is boiled off in two lengths, a large underback must be provided. As it is desirable to prevent the cooling of the wort as much as possible, the underback should always be provided with a steam-coil. In the former case, this vessel should be just sufficiently large to serve the purpose for which it is intended, and the wort should be allowed to remain in it as short a time as possible, for it must be remembered that diastatic action

is continuously proceeding in the wort until it has reached a temperature of 180° or 190° F. in the copper.

Boiling and Hopping.—The mash being completed, the wort, which will have either been run into the copper, or pumped into it as it left the mash-tun, or have been pumped in from the underback, is now boiled for a longer or shorter period.

Objects of Boiling.—The boiling is performed for several objects. The wort as it leaves the mash-tun is swarming with minute organisms and their germs, which, though to a certain extent paralysed by the heat to which they have been subjected in the mash-tun, are not killed. Consequently, if the mash were allowed to remain for any length of time in an unboiled condition, it would soon fall into a state of bacterial fermentation. The prolonged boiling to which it is subjected effectually destroys these organisms and renders the wort sterile. All further diastatic action is also arrested. A considerable portion of the proteïds, since they are coagulable by heat, are thrown out of solution and precipitated, and a further small portion are precipitated by the tannin bodies of the hops. During the boiling the hops are added, the functions of which are to give a pleasant aromatic flavour to the beer, to confer stability on it, and to assist in the precipitation of the proteïd matters of the wort. The wort is also considerably concentrated during the boiling, and this permits a larger quantity of liquor to be used in the sparging and, consequently, a larger amount of extract to be obtained from the malt.

Addition of the Hops.—Much difference prevails as to the time when the hops should be added to the wort in the copper. Some brewers add them as soon as there is a fair quantity of wort in the pan, others just after the wort begins to boil. Probably the most rational method is to add the first portion after the wort has been boiling for ten minutes or a quarter of an hour. By this time a large amount of proteïds have coagulated and been thrown out of solution by the mere action of the heat, and the tannin of the hops is then able to expend its full action upon the portion still remaining in solution; in this way a larger total amount would be precipitated. As the fine delicate aroma of the hop is due to the presence of certain volatile oils, which, to a great extent, are dissipated and lost in the boiling process, it is now customary to add the coarser hops first and the finer ones about twenty minutes or half an hour before turning out, and this modification has much to recommend it. Hops contain various constituents, some of which are much more readily soluble than others, and the presence of both is necessary in the finished beer. But, unfortunately, there are other constituents of a highly undesirable nature also present in hops; these are only brought into solution by prolonged boiling, and, as a consequence, are extracted along with some of the less soluble constituents, which are of a valuable nature. By adding the hops in two portions at different times, this difficulty is to a certain extent overcome. The first portion added is extracted to the fullest extent, and though the undesirable bodies are for the most part

dissolved, yet this long-continued extraction is necessary to secure the solution of the hop resins on which the preservative power of the hops depends. The tannin and the essential oils are also dissolved, but the latter are almost completely lost during the prolonged boil. It is undesirable to have too large a quantity of the hop-resins dissolved in the wort, because they are precipitated in the cooler or in the fermenting vat, and contaminate the yeast. But when a portion of the hops is added at a late stage of the boiling, only small quantities of hop-resin and of the objectionable matters are dissolved, whilst the bulk of the tannin and essential oils is extracted; and as the boiling is now short, these last very valuable constituents are only dissipated to a slight degree. It has even been proposed to add the hops in three portions, the first at the commencement, the second in the middle, and the third near the end of the boil. Naturally, the finest hops will always be added last, since it is the essential oils of these which it is the most desirable to retain. The preservative power of the hops is dependent on the soft resins they contain, and these, on excessive boiling, undergo a chemical change and are converted into bodies of a less soluble nature; hence hops should never be boiled a second time. It is highly probable that the long periods which hops are often boiled at the present time might be shortened with advantage.

When the wort is boiled at twice or three times, or when it is distributed over several coppers, each copper should have its due proportion of hops, calculated on the quantity and respective gravity of the wort of each.

Economy in Hopping.—Among the more recent suggestions for effecting economy in the use of hops, the following may be mentioned:—H. W. Gates (English Pat., 25,774 of 1904) proposes to hop beer in the fermentation vessel by lowering into it a special cylindrical receptacle having perforated walls, filled with raw hops, and pumping the beer from the bottom of the fermentation vessel into the top part of the hop cylinder so that it percolates through the hops. When it is not desirable to pump the fermenting beer, the hops may be packed in a perforated cylinder fitted up so as to serve as a rouser which is stirred about in the beer during fermentation. The hops after being thus extracted are said to be suitable for use in the copper. A. J. Murphy (English Pat., 26,136 of 1904) has also devised an apparatus, consisting of a number of perforated tubes or cylinders arranged in a vertical position between perforated top and bottom plates, and around a central rod, for aërating fermenting liquids and for preparing vegetable and other infusions. This apparatus may be used for hopping beer. For this purpose it is charged with hops and alternately raised and lowered in the liquid.

Hop Separator.—Messrs. Seck Bros., Dresden, manufacture an ingenious apparatus whereby hops may be separated into “leaves” (bracts), ribs and stalks, and “lupulin.” They suggest that the separated “leaves” should be boiled in the copper for about one to one and three quarter hours, or for the length of time usually allowed for whole hops; that the ribs and stalks should be added about 15 minutes before turning out the coppers; and the

“lupulin” (previously soaked in cold water) should be added 10 minutes before turning out the coppers. In order to preserve the delicate aroma of the “lupulin,” it is recommended to shut off steam or withdraw the fires, just before or directly it is added. It is claimed that by the use of the hop separator as above indicated a saving of 10 to 25 per cent. of hops can be effected.

Boiling.—As soon as the wort is in the copper it should be brought to the boil as quickly as possible, for, until a certain temperature is reached diastatic action slowly proceeds, and more and more maltose is formed. The boiling must be thorough; there is probably no more frequent source of unstable beer than insufficient or imperfect boiling.

As to the time of boiling there is much difference in practice. Some brewers only boil for an hour, some an hour and a half, others two hours or longer. Two hours is the most usual time, and will give sufficient cooking. The unnecessary prolongation of the boil causes a useless expenditure of fuel. Whatever length of time is taken for the boil, during the whole of that period the contents of the copper should be kept in a state of vigorous ebullition.

The contents of the copper, unless constantly watched, are so liable to boil over that it is necessary for one person to give his whole attention to the process to see that this does not occur. In order to do away with this constant supervision, it is customary to employ a dome or fountain, which not only prevents boiling over, but also helps to secure an abundant aëration of the wort. Domes and fountains, especially the latter, are objected to by some, since they have a tendency to disintegrate the hops, in consequence of which they do not form such an efficient filter-bed for the wort when it is run out of the hop-back as when they are unbroken. Much has been said on the comparative values of steam and fire coppers. Some contend that a much more vigorous boil may be maintained in a copper heated by direct fire, but the balance of evidence shows that either form is equally good, provided the arrangements for the heating are properly constructed, and the steam-copper is undoubtedly much the more economical in the matter of fuel.¹ Boiling by gas has been introduced into some breweries which are provided with the necessary apparatus for producing what is termed “water gas.” It forms an exceedingly convenient source of heat for this purpose, since its intensity can be so easily regulated.

Boiling at Twice.—In what has gone before, it has been assumed that the whole length, that is, the whole quantity, of the wort is boiled in one copper; but often the copper is not large enough to contain the whole length, and in such a case it has to be boiled at twice, or in extreme cases at three times. Under these circumstances sparging is kept up until sufficient wort is obtained to fill the copper, the taps are then stopped, and the mash allowed to stand on until the contents of the first copper are boiled. It is customary in such

¹ Compare M. J. Cannon, *Journ. Inst. Brewing*, 1906, 12, 39.

cases to add a further amount of hot liquor to the goods in the mash-tun, either by sparging, or underlet, or both.

The next point to be determined is the respective proportions in which the hops are to be added to each copper. It is obvious that if we find the right proportion for the first copper, the remainder will be the proper quantity for the second. The calculation is based on the respective amounts of extract in brewers' pounds in each copper, and if we know the number of pounds in the first copper, that amount deducted from the total number of pounds required for the whole length will be the quantity contained in the second copper wort.

Taking, for example, a brew in which a hundred barrels of beer of a gravity of 21 lb. are to be produced, and to which hops at the rate of 10 lb. for each quarter of malt are to be added, the wort to be boiled in two equal lengths. The total number of pounds of brewers' extract required will be 2100, and if the malt yields 84 lb. extract per quarter, twenty-five quarters of it will be the necessary quantity: 25×84 gives 2100, the total quantity of hops required. On an average the total length of the brew of a hundred barrels, allowing for loss between the copper and the fermenting vessels, will amount to 134 barrels, half of which, or 67 barrels, have to be boiled in each copper length. Say this first length is found to have a gravity of 24 lb., the total number of pounds of extract in the copper will be $67 \times 24 = 1608$ lb. Then—

2100	:	1608	::	250	:	191.4
Total lb. of extract in whole length.		Pounds of extract in first copper.		Total amount of hops.		Amount of hops for first copper

The remainder, $250 - 192 = 58$ lb., will go to the second copper.

Action of the Hop-Tannin Bodies during the Boiling Process.—As has been stated previously, both the hop-tannin and the phlobaphen combine with albumin; consequently, during the boiling process they will enter into combination with the proteid bodies dissolved in the wort. Hayduck found that hop-tannin formed with albumin a compound readily soluble in hot water and slightly so in cold, but that phlobaphen formed an absolutely insoluble compound. In a cold-water extract of barley, which had been boiled and filtered to remove the coagulated albumin, a solution of hop-tannin gave a precipitate which again dissolved on heating, but was reprecipitated as the solution became cold. When this precipitate had been removed by filtration, the presence of tannin could be detected in the clear cold filtrate by ferric chloride, and the presence of albumin could also be detected by tannin, showing that a portion of the tannin-proteid compound still remained in solution. When this solution, which was perfectly clear at the ordinary temperature, was further cooled, it again became turbid. The barley extract behaved in a similar manner without the addition of tannin, though to a less degree; if, after it had been boiled and filtered, it was boiled a second time, a fresh precipitate slowly formed, and this shows that a portion of the proteid matter in solution gradually became insoluble on boiling. When a small quantity of tannin was added to the barley extract, and the precipitate which

formed filtered off, the solution on subsequent boiling, behaved in a similar manner; it was determined experimentally that a small quantity of tannin was carried down with the precipitated matters. When, on the other hand, phlobaphen was added to the barley extract, a precipitate formed which was quite insoluble when the liquid was subsequently boiled. When an excess of phlobaphen was added to the barley extract, all the proteid was completely precipitated, for, neither on the addition of hop-tannin nor of ordinary tannin, was a precipitate produced.¹ From this it follows that the phlobaphen completely precipitates a certain amount of the proteid matters, whilst that portion which combines with the hop-tannin remains in solution during the boiling process; it is partially precipitated as the wort cools, and still further during the fermentation. It was found possible to remove the hop-resins from the scum which formed on the wort, by treating it with ether, and, by afterwards boiling the scum freed from the resins in this way with water to detect the presence of hop-tannin in the solution. Hayduck concludes from these experiments that in the process of boiling, a certain portion of the soluble proteid matters are gradually converted into insoluble modifications, which probably carry a portion of the hop-tannin down with them. These matters cannot, however, be completely separated by boiling, for their presence could be invariably detected in beer by tannin, hop-tannin, or phlobaphen, though beers which were heavily hopped contained smaller quantities of albuminoid matter uncombined with tannin. He considers that the hop-tannin and phlobaphen, since they remove a portion of the proteid matter from beer, act indirectly as preservative agents, but that they have no direct influence. He had previously shown that hop-tannin exercised no action in arresting the growth of the lactic bacteria, and that an aqueous extract of hops, from which the resins had been removed by treatment with ether, had just as little effect.

From an experiment made by Briant and Meacham,² the quantity of albuminous matter removed by boiling with hops is very small. Two brews were conducted under similar conditions, the sole difference being that in one (A) hops were used at the rate of 8 lb. per quarter, in the other (B) at 16 lb. The result was as follows:—

Soluble Albuminoids in Dry Extract.

	Copper Boiling.	Copper Out.	Albuminoids Precipitated.
	Per cent.	Per cent.	Per cent.
Experiment A	5.93	5.75	0.18
Experiment B	6.01	5.83	0.18

¹ Probably the beneficial effect of a prolonged vigorous boil may be due to the conversion of the hop-tannin into phlobaphen.

² *Transactions of the Institute of Brewing*, 6, 160.

Turning Out.—After the wort has been boiled for a sufficient length of time it is “turned out”—that is, its contents are run out into the hop-back. The hops gradually settle down to the perforated bottom of this vessel, where they form a layer of filtering material, and as the wort runs off, hold back the various matters which have been precipitated during the boiling process; the wort consequently passes away in a bright and clear condition. Some breweries are not provided with a hop-back, but have a strainer fixed in the copper which retains the hops; this is a faulty arrangement, since under these conditions the wort only flows away very slowly and much waste of time ensues.

Cooling.—After a sufficient length of time has been given for the hops to settle in the back, the wort is either run or pumped on to the cooler, and as it is there exposed to the air in a thin layer, it rapidly loses heat. The wort adhering to the spent hops is recovered either by pressing or by sparging them; the latter plan is to be preferred. The cooling effect of the air, is sometimes increased by a fan placed in the middle of the cooler, which, when set in motion, induces powerful currents of air which are wafted over the surface of the wort. The wort, while on the cooler, is supposed to take up a quantity of oxygen, which, as was shown by Pasteur, enters into chemical combination with some of its constituents. This combined oxygen exercises a profound influence on the readiness with which the wort undergoes subsequent clarification. In a wort which has been well aërated the precipitated particles agglomerate into coherent masses, which subside readily, whilst in an unaërated wort they remain suspended in a state of fine division. The wort also becomes darker in colour under the influence of the combined oxygen; the darkening due to this cause, however, entirely disappears during the fermentation. L. Briant¹ brings forward facts which constitute a strong argument in favour of hot aëration, but he points out that it does not necessarily follow that bright beers cannot be produced without coolers, since many breweries, producing bright beers, have no coolers. He shows that oxygen is absorbed most readily at a high temperature, and that some absorption takes place in the copper (especially if it be an open one), but that a larger amount of absorption occurs in the hop-back. Briant condemns the injection of air into the wort in the hop-back as it disturbs the filter-bed, and he states that the injection of air into the wort in the rising main has proved most effective in certain cases; whilst the same may be said of injection in a deep or closed back. The wort, during its passage through the cooler, deposits much matter, which is known as “cooler sludge,” and this should not be allowed to be carried away with the wort. Wort should not be allowed to stay on the cooler for a longer period than is necessary to bring its temperature down to 140° F., for, if allowed to remain for any length of time at a temperature a few degrees below this, it is liable to undergo changes of an objectionable character, the result of which is the production of a beer of a less stable

¹ *Journ. Inst. Brewing*, 1904, 10, 286.

character. As soon as, or a little before, the wort has arrived at 140° F., it should be passed on to the refrigerator.¹

Refrigerating.—In passing over the refrigerator, the wort takes up a further quantity of oxygen; this does not, however, chemically combine with it, but simply enters into a state of solution. This dissolved oxygen is of great importance in stimulating the yeast and starting a vigorous fermentation. Since vertical refrigerators afford the fullest exposure of the wort to the air, they are to be preferred to the other forms which do not possess this important advantage. The rate at which the wort passes over the refrigerator is so regulated that it leaves the apparatus at the temperature required for commencing the fermentation, which, as a rule, is 60° F., or two or three degrees below.

Employment of Germ-Free Air.—In recent times it has been proposed to carry out the operations of cooling and refrigerating in such a way that the wort is kept entirely out of contact with the outside germ-laden atmosphere, and only allowed to come in contact with air which has been deprived of its germs by filtration. Consequently, wort treated in this manner reaches the fermentation vessels in as sterile a state as it left the copper, and this is undoubtedly a step in the right direction. The wort is most liable to be contaminated by living germs during its passage over the refrigerator, for its temperature when on the cooler is so much higher, that any germs which fall in are either destroyed or have their vital energy considerably lowered. The organisms which gain access to the wort on the cooler are for the most part bacteria, as yeasts are killed at a much lower temperature than the latter. On the other hand, contamination by wild yeasts as well as bacteria occurs on the refrigerator.

Collection of the Wort.—After refrigeration the wort passes either into the collecting vessel or, more generally, it is run directly into the fermenting tuns. In either case it is here that the Excise officer takes a note of its quantity and gravity, and upon the calculation made from these data the amount of duty payable is based.

Excise Charges.—The duty on beer is levied at the rate of 7s. 9d. for each barrel at a specific gravity of 1055, an allowance of 6 per cent. being made for waste and loss during fermentation. This allowance is not reckoned off each day's brew, but is deducted at the end of the month, when the book is made up for payment. Consequently beer, at whatever gravity it is brewed, has to be reduced to its equivalent in gallons at the standard gravity of 1055. The calculation is effected in the following manner. The number of gallons in a brew is ascertained from the dip,² and the figure thus obtained multiplied by the excess of the gravity over 1000. Suppose we have a brew of 3600

¹ Compare R. L. Siau, *Journ. Inst. Brewing*, 1906, 12, 119.

² All fermenting vessels are gauged by the Excise officers, *i.e.*, the number of gallons corresponding to each inch of depth ascertained. The number of gallons of wort actually contained in the vat is found by the dipping-rod, which is a kind of boxwood rule divided into inches.

gallons of sp. gr. 1060; then $3600 \times 1060 - 1000 = 216,000$. This figure divided by 55, the excess of the standard gravity over 1000, gives $216,000 \div 55 = 3927.27$. Therefore, excluding the decimals, 3927 gallons of wort at 1055 sp. gr. are equivalent to the 3600 gallons at 1060 sp. gr., and this former figure (3927) is entered into the day-book. At the end of the month the amounts for each day are added together, the allowance of 6 per cent. for waste deducted, and the remainder brought to barrels by dividing by 36. Thus, suppose the amount brewed in one month was equivalent to 125,640 gallons at standard gravity (1055); this multiplied by 0.06 would give the 6 per cent. to be deducted for waste, which would be 7538.4, and $125,640 - 7538 = 118,102$, which is the gross number of gallons for which duty would be charged. The number thus obtained is then converted into barrels by dividing by 36; thus $118,102 \div 36 = 3280$ barrels and 22 gallons. The duty is not collected on the 22 odd gallons, but they are carried over as "odds" to the next monthly account. The duty per barrel, 7s. 9d., multiplied by 3280 gives £1276. From this it will be seen that the brewer, when not paying on materials, pays duty on the weight of extract yielded at the rate of 7s. 3.42d. for each 19.8 lb. of brewer's extract, since each barrel of beer at a specific gravity of 1055 contains this amount of brewer's extract, and 7s. 9d. less the allowance of 6 per cent. equals 7s. 3.42d.

If the brewer does not obtain four barrels of wort of specific gravity of 1055, less 6 per cent., from each quarter, or 336 lb. of malt or its equivalent, the charge, instead of being levied on the wort, is levied on the materials used, and in this case a higher rate is paid. A quarter of malt is supposed to yield 4 barrels of beer at 1055 or 19.8 lb. per barrel, so that $19.8 \times 4 = 79.2$ lb. per barrel is the standard extract for a quarter of malt. From the amount of the charge obtained in this way the 6 per cent. for waste is deducted as when the charge is made on the wort. 224 lb. of cane sugar are deemed by the Excise authorities to be equivalent to a quarter of malt weighing 336 lb., and similarly 256 lb. of glucose or of invert sugar, or of flaked maize or rice, 272 lb. of No. 1 syrup weighing 14 lb. per gallon, or 328 lb. of No. 2 syrup weighing 13 lb. 2 oz. per gallon. It is only, however, with very badly arranged plant, or with the use of very indifferent materials or want of skill in working, that this condition of things can arise.

Extract per Quarter Actually Yielded.—The brewer should never omit to ascertain from day to day the actual amount of extract which his goods are yielding, as this at once affords valuable information as to the manner in which work is being carried on. To do this, the number of barrels in each brew is multiplied by the gravity expressed in brewers' pounds, and from this is deducted the amount of brewers' pounds obtained from sugar or syrup. The figure thus obtained is then divided by the number of quarters of malt used. For instance, if in a brew in which 25 quarters of malt were used, and 14 cwts. invert sugar yielding 35 lb. per cwt., 125 barrels of wort were produced of a specific gravity of 1055, the total number of pounds of brewers' extract contained in this would be 125 multiplied by 19.8 (this latter

figure being the equivalent of sp. gr. 1055, *i.e.*, $1055 - 1000 \times 0.36 = 19.8$). The sum obtained would be 2475 lb. of brewer's extract, and of this 490 lb. were obtained from sugar; deducting this, $2475 - 490 = 1985$ lb. were obtained from the malt; since 25 quarters of malt were used, then $1985 \div 25 = 79.4$ lb. per quarter.

Percentage of Extract upon the Malt.—As there is some doubt concerning the actual amount of solid matter in solution which corresponds to 1 lb. of brewers' extract, or to a degree of specific gravity, it is impossible to give an exact method of finding the actual amount of solid matter dissolved from the malt. The multiplying factor generally employed is 2.59, which is based on the assumption that when 1 lb. of malt-extract is dissolved in water and the amount made up to 10 gallons, the specific gravity is raised 3.86 degrees (water = 1000). Though the specific gravity of a solution of cane-sugar is raised by 3.86 for every gram per 100 cc. it is not so in the case of malt extract solids, one part per 100 volumes of the solution of which, according to O'Sullivan, raises the specific gravity 3.95°, and, consequently, the factor would be 2.532. Probably the nearest approximation to the truth is obtained by assuming that 1 part by weight of solid matter in 100 volumes of malt extract (*i.e.*, one gram of solid matter in 100 cc., or one pound in 10 gallons) raises the specific gravity by 4 units.¹ This materially simplifies calculation, and requires the reciprocal factor 2.5.

As an example of the differences which may arise by the employment of different solution factors it may be pointed out, that if a malt yield 85 lb. extract, and if the cane sugar factor be employed, the actual matter dissolved from a quarter of malt will be $85 \times 2.59 = 220.15$ lb.; if the factor 4, $85 \times 2.5 = 212.5$. From this the percentage yield of malt is obtained by the proportion sum 336 : 100 :: 212.5 : 63.2, which is the percentage when the factor 2.5 is employed.

FERMENTATION.

During the process of fermentation the wort is submitted to the action of yeast, through the influence of which considerable portions of its saccharine constituents are converted into alcohol and carbon dioxide; it is here that the liquid first assumes its true character of an alcoholic and fermented beverage. The methods in which this part of the operation of brewing is conducted vary considerably, though all have one common object. They may be roughly classified under the following three heads—the *cleansing* system, the *skimming* system, and the *stone square* system.

Addition of Yeast to the Wort.—This is technically known as "pitching." In many breweries the usual custom is to add the pitching yeast, the proper quantity having been previously ascertained by weighing, to the whole of the wort after it has been collected in the fermenting tun. This plan cannot be recommended, since it delays the active reproduction of the

¹ Malt extract contains, besides maltose and maltodextrins, mineral matters which have higher solution factors than the carbohydrates.

yeast, and it should be the brewer's object to promote a rapid development of yeast in the wort, for the sooner this is brought about the less chance foreign organisms have of obtaining a foothold. It is therefore much better first to run down a small portion of the wort at a temperature of from 65° to 75° F., and to mix the yeast with this. In this way a rapid and vigorous growth of yeast is secured from the onset, and the reproduction of any bacteria, should these happen to be present, is effectually held in check. The remainder of the wort is then run in at a slightly lower temperature than that which the whole bulk is to have when collected, so that at the finish the gyle may be at the proper heat. The wort, while being collected, is roused at frequent intervals in order that the yeast may be evenly diffused through it.

Choice of Yeast for Pitching.—The fitness of yeast for this purpose is determined by its general and microscopical characteristics. With regard to the former, it should possess an agreeable taste and odour, and, if derived from ale wort, should be of a pale yellow colour. The best yeast for pitching is obtained from medium gravity worts (18 to 20 lb. gravity), which are not too heavily hopped. In London the yeast obtained from the porter is held in high esteem. The first heads of yeast thrown off during fermentation are always very dirty, since they bring up with them much of the substances precipitated during cooling, such as the compounds of hop-tannin with the albuminoid matters of the malt, hop-resin, &c., and as they are quite unfit for pitching purposes, they are thrown away. The middle skimmings, which yield the best pitching yeast, are preserved. The last skimmings are also rejected, because the yeast finally thrown off is not so strong and vigorous as that of the middle skimmings. Much stress has been laid upon the proper consistency of the pitching yeast, and it has been the rule only to use yeast from which the beer has been allowed to drain to such an extent that the yeast is left in a firm semi-solid pasty condition, without any tendency to sloppiness. Young yeast, which is generally thin because sufficient time has not elapsed for the beer to drain away from it, should not, if good in other respects, be rejected on account of its thinness; naturally a little more must be added to allow for the beer it contains. But thick yeast, which has become sloppy through age, should never be employed, for sloppiness in this case is an indication that putrefaction has already commenced. That these external characteristics may be at times delusive is shown by the No. 1 Carlsberg bottom yeast, which, though it has yielded excellent results during a long series of years, is said to be wanting in all those external characteristics which are usually regarded as of importance. As to the age of yeast, in the opinion of Southby and other reliable authorities, the younger it is the better; in extreme cases it should never be employed when more than a week old, and this during the coldest months in the year; during the hottest it cannot be safely used when more than four days old. Much, however, depends on the method of storage adopted. Yeast tanks should be shallow and furnished with attemperators.

The yeast used for pitching should be frequently submitted to microscopical

examination. When placed under the instrument, the cells should appear uniform in shape and size, detached from one another, plump, and with sharp, well-defined borders. The protoplasmic contents should be transparent, with cloudy markings here and there, the vacuoles clear and of moderate size. Very young cells have no vacuoles, and their contents are quite clear and transparent; as the cells mature the vacuoles increase in size, and the cell-contents become granular. The cells shrink in size as their age progresses, their walls become thickened and wrinkled, and their protoplasmic contents exceedingly granular. When this stage is reached, the cells are either quite dead, or so deteriorated as to be useless; consequently, a sample which contains even a trace of such cells should never be used for pitching purposes. The number of cells actually dead may be easily ascertained by running under the cover-glass a weak solution of one of the aniline dyes, such as methylene blue, which immediately stains the dead cells a deep blue colour.

Bacterial organisms should be carefully looked for during the microscopical examination, several fields of the same yeast being systematically gone over, and the number of bacteria present in each noted. Good and reliable yeast ought to be practically free from these organisms.

Hansen strongly advises the employment, for pitching purposes, of that portion of the yeast which is produced in the earlier stages of fermentation, since it is the least likely to be contaminated with wild yeasts.

Quantities of Yeast required for Pitching.—The quantity of yeast to be used for this purpose is generally estimated by weighing, and, when speaking of pounds of yeast, it must be understood that this refers to good thick stiff yeast. Should the yeast be insufficiently drained and contain beer, an allowance must be made for this, and proportionately more yeast added. The quantity of yeast required for pitching a tun is usually calculated at so many pounds of yeast per barrel of wort, and its amount varies with the different classes of beers, proportionally more being necessary for high gravity and for heavily hopped worts. From $1\frac{1}{2}$ lb. to 2 lb. per barrel will be necessary for a beer of 18 lb. to 20 lb. gravity, brewed with 5 lb. to 6 lb. of hops per quarter of malt. A 24-lb. wort, with 14 lb. to 16 lb. of hops per quarter, will require from $2\frac{1}{2}$ lb. to $3\frac{1}{2}$ lb. of yeast per barrel, and worts stronger than these some $\frac{1}{2}$ lb. per barrel more. The salts in the brewing water, when their amount is not inordinately large, seem to have little or no effect upon the quantity of yeast required; but when antiseptics are added to the wort, they exercise a retarding action on the yeast, and hence necessitate its addition in slightly larger quantities. This is especially true of salicylic acid and the bisulphites, though it is questionable if the monosulphites have much action in this respect. In those systems of fermentation in which a considerable amount of rousing and aëration takes place, the conditions are especially favourable to the growth and multiplication of the yeast, and consequently less pitching yeast is required. Yeast, when continuously passed through several gyles of strong wort, gradually becomes more and more sluggish in its action, probably from being surfeited with

nitrogenous matter. When frequently cultivated in heavily hopped worts, the surface of the cells acquires a coating of hop-resin, which naturally interferes with the fulfilment of its proper functions.

Changes of Yeast.—In some breweries the yeast, after being in use for a longer or shorter period, commences to evince signs of degeneration; it gradually becomes slower and slower in its action, and performs its work generally in an unsatisfactory manner. When this happens a change of yeast has to be obtained from some other brewery. The degeneration is in many cases brought about by bacterial contamination, and in this case a microscopical examination at once reveals the cause of the trouble. Yeast degeneration of this kind can only occur where brewing operations are carried on without a proper regard to cleanliness; it is often associated with a dirty condition of the wort pipes and other communications. In other cases, the cause of the degeneration is much more obscure, and probably depends upon the yeast not finding a sufficiency of some substance or substances in the wort which are necessary for its perfect nutrition.¹ Thausing lays the blame chiefly on inferior malt or faults in the mashing process. Whilst some brewers find it necessary to obtain a change of yeast every few weeks or months, there are others who are able to work with the same yeast for almost unlimited periods; and, granting that the yeast invariably receives a proper supply of all the substances necessary for its nutrition, and that the brewing operations are conducted with a due regard to cleanliness, it is difficult to understand why this should not be more universally the case. The Carlsberg bottom yeast, No. 1, which has been used at the Carlsberg brewery continuously since 1883, is an excellent instance of this.

In making a change of yeast, the fresh supply should be, if possible, secured from a brewery working on different lines, and, if possible, from one where the worts are entirely produced from malt. Too great dissimilarities in the type of yeast should be avoided. But here it should be mentioned that although the slow-fermentation yeast used in the stone square system can be used in an open vessel if pumped and roused,² the converse is not the case; yeast from no other system of fermentation can apparently be used satisfactorily in a stone square. Any new supply should be rigorously examined before being taken into actual use; and, when a change is being

¹ A remarkable instance of this occurred at the Kallinkin Brewery, at St. Petersburg, where Hansen's system of single-cell yeast is employed. A yeast which had yielded eminently satisfactory results for two years began to degenerate, and became at last unusable. The cause of the mischief was found to be the absence of sufficient lime in the worts, the water employed at the brewery, both for malting and brewing purposes, only containing 0.91 grain of lime (CaO) per gallon. The addition of gypsum to the steeping water and mash liquor brought back the yeast to its former pristine vigour, and restored the proper amount of lime to its ash, which was at first 4.48 per cent., but which had fallen as low as 0.54 per cent.

It was found experimentally that a vigorous yeast introduced into a wort from which the lime had been almost completely removed by dialysis caused a "bladdery" fermentation.

² Compare Rogerson, *Journ. Inst. Brewing*, 1906, 12, 518.

effected, it is well to keep some of the old stock in hand, as there is always a possible chance that the new may turn out worse than the old.

Making a change of yeast is always a matter of great uncertainty; matters may be improved by the new supply, or the change may prove for the worse. This uncertainty is removed, however, when Hansen's reformed system is adopted. In a brewery supplied with a pure yeast propagating apparatus, the brewer, when his stock of yeast begins to show signs of degeneration, instead of having to seek a change of yeast from another brewery, with all its consequent uncertainties, simply turns to his own apparatus, with the full assurance that he will there find a supply of yeast of exactly the same nature and character as the one he has been using. The limitations of this system have, however, been explained on pp. 386-388.

Temperature at which Fermentation is Conducted.—This varies somewhat with the different classes of beer. The weaker qualities, such as those of a gravity of from 18 to 20 lb. are generally started at about 58° to 60° F., and are not allowed to rise beyond 70°. But the better plan is not to permit them to rise beyond 66°, since a temperature above this is liable to encourage the growth of bacterial organisms. With stronger beers greater latitude is often allowed; they are started at from 56° to 58° F., and allowed to run up to 75°, since the larger amount of alcohol formed, coupled with the more vigorous fermentation in a strong wort, serves to keep bacteria in check, but even these are all the better for being kept down to 66°. Low fermentation temperatures conduce to the production of beers having a fine flavour; moreover, they contain larger quantities of carbon dioxide in solution, and are more stable. Slightly higher temperatures are admissible in the case of black beers; the brown and black malts, to which these particular types of beer owe their distinctive characteristics, appear to exert a certain antiseptic influence on the worts. Where the plant is provided with powerful attenuators the fermentation may be commenced at a higher temperature and confined within narrower limits, between say 62° and 65° F., with good results.

Dressing.—When the fermentation is sluggish and the yeast crop does not develop with its usual vigour, the heads being persistently discoloured with hop-resin which ought to have been brought up by the first heads, it is usual to dress the gyle. The old-fashioned way was to take 1 lb. of wheat-flour and 4 ounces of salt for each ten barrels of wort in the gyle, and to mix these ingredients well together on the surface of the fermented wort, after which a thorough rousing was given. It was formerly supposed that the stimulative action of the dressing on the yeast was due to the albuminoids of the wheat, which were dissolved by the salt. Now it is known that the effect is caused by the diastase present in the wheat-flour, and that in those cases where dressing is beneficial, the cause of trouble is too high a percentage of the more unfermentable carbohydrates in the wort, which, after being degraded by the diastase of the flour used in dressing, are rendered amenable to the action of the yeast. It is now more customary to use the more diastatic malt-flour instead of wheat-flour, and this without the addition of

salt. Dressing is always objectionable, because a number of bacterial organisms and their germs are introduced into the wort; consequently, it should only be resorted to in very exceptional circumstances.

Appearance of the Heads of Yeast during Fermentation.—In all normal fermentations the yeast thrown up on the surface of the fermenting wort, technically called the head, undergoes certain characteristic changes, and considerable information may be obtained as to the way in which the yeast is fulfilling its functions by carefully observing the heads. When the wort has been pitched at the usual temperatures (58° to 60° F.), the commencement of fermentation may be detected in two or three hours by the appearance of small bubbles of carbon dioxide which rise to the surface. In another two or three hours froth begins to form round the sides of the vessel, and this gradually extends over the whole surface and increases in volume, until what is termed the “cauliflower” stage is reached; this then gradually passes into the “rocky head” stage. The heads go on steadily increasing for a time, and often attain a height of three or four feet above the surface of the wort. The more or less frothy head now commences to fall and the “yeasty head” commences to form. This is in a constant state of motion from the continual formation and bursting of the large bubbles of gas which is now being rapidly disengaged. With the commencement of the formation of the yeasty head, what is known as “skimming-point” is reached, the normal time for this being about forty-eight hours from the time of pitching. The gravity of the wort will by this time, according to circumstances, have been reduced to from one-half to two-thirds of its original gravity. It is at this point that the separation of the yeast from the beer begins in the cleansing and skimming systems, and it is also the point at which the treatment of the wort on the different systems diverges.

The Cleansing System.—Probably this is the oldest system of all, and is the survival of the old home-brewing, where the same tub did duty for mash-tun and fermenting vessel, the beer being cleansed in the casks in which it was eventually stored. This primitive method of cleansing is still employed in some breweries, the wort, after being fermented for about forty-eight hours, being run into the trade casks and there cleansed. Later on larger casks, holding from two or three barrels and known by the name of “loose pieces,” butts, puncheons, and pontos came into use (see pp. 504, 505). The loose pieces were mounted on a large wooden trough called a stillion, and from this arrangement doubtless arose the Burton union system. In the cleansing system the wort is pitched at from 56° to 60° F., and the fermentation allowed to proceed until its gravity has been reduced to about one-half, by which time its temperature will have risen to about 70° , a point generally reached in thirty-six to forty hours from the time of pitching. The half-fermented wort is then run off into the cleansing casks. This distribution of the wort into much smaller quantities serves to keep its temperature down; consequently, when the temperature of the fermenting-room does not exceed 45° or 50° F., the contents of the cleansing casks never rise higher than 70° with worts of medium strength, or a

few degrees higher with very strong worts. In the warmer months of the year, where the cleansing is performed in loose pieces, there is a danger of their temperature rising too high ; consequently it is customary during the hotter months of the year to run off the wort from the fermenting vat at a somewhat earlier stage of fermentation before it has reached a temperature of 70° F. The Burton unions are provided with attemperators, and these are used in the summer months for keeping the wort at the proper temperature.

The yeast which is removed during the process of cleansing carries away with it a certain amount of beer ; and unless some automatic arrangement, such as the Burton unions are provided with, is employed, the casks have to be replenished by hand, or, as it is technically termed, "topped up," at frequent intervals. If the casks are not kept completely full, a portion of the yeast, instead of being ejected through the bung-hole, sinks to the bottom. The first topping up takes place in about four hours after the beer has been turned into the cleansing vessels, and is repeated every three hours while the fermentation is vigorous ; when this slackens the filling up is performed at less frequent intervals. The beer used for this purpose should be as free from yeast as possible.

The Skimming System.—In this the fermentation is started in the same way as in the cleansing system, but when the skimming-point is reached (p. 535), the wort, instead of being run off into cleansing casks, is well roused. As soon as the head begins to assume a distinct yeasty character it is skimmed off every six hours ; in some breweries oftener. This may be effected by hand or by any form of skimming apparatus with which the tun is provided. The wort which passes off with the yeast should be freed from the latter as soon as possible and returned to the vat. As in this system no subdivision of the wort takes place, the regulation of its temperature is entirely dependent upon the attemperators ; consequently these should be of ample power. When the temperature of the fermenting wort has risen to about 59° or 59.5° F., the attemperator is started slowly, and the flow of water through it is so regulated that the heat is allowed to rise half a degree every three hours. When the temperature has reached 65° or 66° F., the attemperator is set more vigorously into action, and any further rise in temperature prevented. When the fermentation on approaching completion begins to slacken, the flow of water in the attemperator is still further increased, and the temperature of the beer brought down, in the hotter months to 60° F., in the colder to a somewhat lower degree.¹ This cooling may, contrary to the opinion commonly held, be rapidly effected without any danger of the beer acquiring a yeasty flavour. The skimming is kept on until it is judged that the beer will be able to throw up just one more head of sufficient thickness to protect it from aerial contamination. The right point to stop skimming is found by pushing a small portion of yeast on one side and examining the surface of the beer thus exposed ; when

¹ The practice as to the temperature to which the wort is permitted to rise during fermentation, as also the time at which the attemperator is started, differs in nearly every brewery. Some brewers allow even their low gravity beers to rise to 72° F.

this appears black and clear, denoting that there is scarcely any more yeast in suspension, the skimming is stopped, and the head which subsequently forms is allowed to remain untouched. Should the beer appear brown and turbid it is a sign that a considerable quantity of yeast is still in suspension, and skimming must still be kept on. It is better to err on the side of leaving too thick a final head of yeast than the reverse.

The Dropping System.—This system was devised by the late William Garton. It is a modification of the skimming system, the beer is collected in the upper vat and pitched as in the skimming system. After the fermentation has nearly reached skimming-point (p. 535), it is run down into the lower vessel, where it is treated exactly as in the skimming system. During the process of dropping, the wort is thoroughly roused and aerated, and leaves behind in the upper vessel much of those precipitated matters which are collectively termed "slummage."

The Stone Square System.—This system, which is exceedingly popular in Yorkshire and the North of England, yields beers which drink very full for their gravity, and which, since they retain large quantities of carbon dioxide, are full of life. The yeast employed is of a peculiar type, is exceedingly slow in its action, and requires the frequent rousings and aëration which are peculiar to the system to compel it to fulfil its functions properly. The wort is pitched at a temperature of 58° to 59° F., with a smaller proportion of yeast than under the other systems, from 1 to 1½ lb. per barrel being sufficient. The yeast is generally added to the wort when the whole of it is in the square; but a much better plan is to add it to the first portion of wort, running this down at a temperature slightly higher than the remainder, for the reasons already stated (p. 53). The square is pitched in the following manner:—The valve at the upper end of the organ-pipe is closed and a portion of wort run into the upper chamber; the yeast is then thoroughly roused in with this, the valve opened, and the mixed yeast and wort allowed to flow into the lower chamber. When the whole of the wort has been collected and pitched, it is left undisturbed for thirty-six hours, at the end of which it should have risen to about 62° F. It is now roused for the first time, the rousing being repeated every two hours during the next twelve hours, at the expiration of which (forty-eight hours after pitching) pumping commences. Before starting the pump the valve of the organ-pipe is shut, and as much wort pumped into the upper compartment as is delivered by fifteen strokes of the pump; it is then well roused, so as to mix in the yeast which has risen through the man-hole, after which the valve is opened and the wort allowed to flow back into the lower chamber. Pumping and rousing are repeated every two hours, the number of strokes of the pump being increased at each repetition of the operations, beginning with fifteen strokes for the first pumping, and increasing by ten at each repetition. This is continued until the wort has reached a gravity some 1 or 1½ degrees higher than that required at the finish. During the whole period of fermentation the temperature of the wort is kept within the necessary limits by means of the attemperating jacket. When the pumping

and rousing stage is passed, the organ-pipe valve is closed, and the yeast which rises through the manhole removed every four hours. After each removal the valve is opened for a short time to allow the beer which has drained from the yeast to run down into the lower compartment. When, by observing the surface of the beer as in the skimming system, the yeast appears fully removed, the temperature of the beer is gradually brought down by attempting to 60° F., or a little lower. The manhole is then covered with the cap, and the contents of the square allowed to remain undisturbed for two days, by which time the beer is ready for racking into the trade casks.

Settling.—When the primary fermentation in the fermenting vat is completed, the beer is allowed to remain at rest for twenty-four hours or longer, in order that it may deposit the bulk of the yeast which still remains in suspension. This part of the process may take place in the fermenting vat itself, or in the settling back, where this vessel is employed. The latter vessel is by no means so frequently used as it was formerly, for it is found that the less the beer is moved about and exposed to the air at this particular stage the better.

Racking.—As soon as the beer has become sufficiently bright it is run off, or, as it is termed, “racked off,” either into the store or trade casks, each of which, before being filled, is carefully examined to ascertain that it is perfectly dry, clean, and sweet. Care is taken that only the clear beer passes off, which object is secured by having an arrangement of taps at different levels, or by a tap with an internal sleeve. For conveying the beer from the vat to the cask a leather or india-rubber hose is employed, one of its ends being attached to the tap in the vat, and the other provided with a metal thimble or a piece of metal pipe. The object of the latter is to keep the end of the racking-tube close to the bottom of the cask, so that violent agitation of the beer, which causes the excessive frothing known as “fobbing,” may be avoided. Each cask, after being filled, is temporarily closed with a bung and removed to some convenient place, where, after a period of rest, it is filled up by hand, the shive inserted, and the cask taken to the store cellar.

Dry Hopping.—It is usual to add a small quantity of fresh hops to beer at the time of racking; this is known as “dry hopping,” or “hopping down.” Hops of the best quality only are used for this purpose, such as Goldings or Worcesters, or the finest varieties of foreign hops, the former being added preferentially for their fine flavour and aroma, the latter for their preservative powers. The hops used should be absolutely free from yeasts, mould fungi, and bacteria which might cause disease in the beer, and they should be as little disintegrated as possible. The quantities employed vary from half a pound to one pound per barrel, or more for very strong ales. The amount required for each cask is weighed out and introduced into the empty cask by means of a wide funnel through which the hops are pushed with a short wooden rod, care being taken that the hops are simply loosened, and not broken into fragments. Hops added in this way confer additional

flavour and aroma on the beer, since the whole of the essential oil which they contain is retained. They also assist in promoting condition, which is so highly conducive to stability. As was shown by Brown and Morris, hops contain a diastatic enzyme, which degrades some of the lower malto-dextrins, and converts them into a form in which they can be fermented by the yeast which is always present in traces in every cask of beer.¹ Hops also assist the clarification since they form points of attraction for fine particles of matter in suspension, just as do the wood chips or aluminium strips used by the German brewers.

Secondary Fermentation.—Beers when stored in vat or cask for any length of time, pass, under normal conditions, into a very slow fermentation which, to distinguish it from that which takes place in the fermenting vat, is termed “secondary.” Often the secondary fermentation becomes unduly excited; the beer is then said to “fret” or “kick up.” Such a state of matters points either to faulty goods or defective manipulation, or both, in the previous operations. As a rule this is due to the recurrence in cask of the primary fermentation and not to true secondary fermentation. During secondary fermentation the more resistant maltodextrins are gradually and slowly degraded by the hydrolytic action of the special yeasts concerned, assisted by that of the diastase of the dry hops when these are added at the time of racking, and perhaps also to some extent by the carbonic acid existing under pressure. The maltodextrins, when broken down in this way, become amenable to the fermentative power of the yeast, and by their fermentation keep the beer constantly charged with carbon dioxide, in which condition it is much better able to resist the attack of disease ferments, if such organisms should happen to be present. As carbon dioxide holds the majority of disease ferments in check, saturation of the beer with this gas is an important factor in promoting its stability. The carbon dioxide produced under these circumstances is evolved in the nascent state and under pressure. Consequently, a portion of it combines with water to form the carbonic acid (H_2CO_3), which gives the beer that peculiarly sharp, stinging sensation on the palate, especially noticeable in bottled beers which have been stored for some time. Certain compound ethers or esters are also formed on prolonged storage, and these are much concerned in the fine flavour characteristic of fully ripened and matured ales (see p. 540). But the demand for beers of this class seems to be gradually passing away; the tendency nowadays is towards less ripe and mature ales, and consequently the period of storage is often reduced to weeks, or is even omitted altogether. In the former case, the secondary fermentation, which, as R. L. Siau (see p. 387) has pointed out, is then really a continuation of the primary fermentation, is hurried on as rapidly as possible by frequently rolling the casks, and thus keeping the yeast in suspension. In the latter case, the beers are sent out immediately after racking, dry hopping, and fining; consequently, what little fermentation

¹ Wahl (*Der Braumeister*, 1889, p. 307) found 295,000 yeast cells in each cubic inch of a bright finished beer, and in a brilliant sample 82,000.

there is takes place in the publican's or customer's cellar. The worts for such beers contain an abundance of readily fermentable matters, which disappear during the primary fermentation, and also some of the very low maltodextrins, which, though slowly fermentable bodies, pass off with sufficient rapidity to keep the beer charged with gas.

Priming.—Of late years it has become customary to “prime” beers which are to be quickly consumed immediately before being sent out, and in this way to induce a sort of artificial secondary fermentation—really a continuation of the primary fermentation (see last paragraph)—in them. This is effected by the addition to the beer of a strong solution of one of the readily fermentable sugars, such as cane-sugar or invert sugar, or of dextrin maltose (see p. 89). Priming is exceedingly useful in many cases, but the condition which it induces is of a far more transitory character than that obtained by allowing a beer which has been brewed on correct lines to mature slowly by the natural process.

The syrups used for priming are made of a specific gravity approaching 1150°, but must not exceed this, since it is the highest gravity that the instrument used by the Excise officers is capable of verifying. But in order to secure the best results the syrup should be as strong as is permissible. It must be kept in a special vessel properly gauged and marked.

The Yeast of the Secondary Fermentation.—It will be remembered that Brown and Morris (pp. 159–160) found that when a wort was pitched with the *Saccharomyces cerevisiae* only a certain portion of its extract, which, under their theory, they assume to be free maltose, was fermented, but that on the addition of one of the wild yeasts, such as *S. ellipsoideus*, a further portion of the constituents of the wort was fermented away. From this they concluded that the secondary fermentation could only be effected through the agency of wild yeasts. They suggested that these obtained access to the wort during the time it is exposed to the air when on the cooler, refrigerator, &c.; and this view has met with many adherents. On the other hand, it has been stated that worts which have not been allowed to come in contact with anything but germ-free air after leaving the copper, and which have been seeded with a pure culture of *S. cerevisiae*, pass through the secondary fermentation just as readily as those brewed in the ordinary way. From the evidence we now have, however, it is probable that the conditioning which has been observed in the case of running comparatively quick-draught ales brewed with single-cell yeast is not to be ascribed to a true secondary fermentation, but to a survival of the primary fermentation which invariably occurs to a greater or less extent in all beers after racking, and may be increased by the addition of priming or by racking at high gravities. R. L. Siau, who has published the latest experimental evidence on the use of pure yeast in British brewing, has arrived at conclusions substantially identical with the above (see p. 387). The observation of N. H. Claussen that the secondary fermentation of British beers is due to a species of *torula* has already been dealt with (see p. 388).

Secondary Volatile Products of Fermentation.—As is well known, in all fermentations approximately 5 per cent. of the sugar is converted into glycerol, succinic acid, formic and acetic acids, hydrogen, the higher alcohols, acetal, formaldehyde, compound ethers or esters, and several other bodies, which collectively may be termed the by-products of fermentation. There can be no doubt that distilled spirits, such as cognac, whisky, &c., owe their distinctive flavours to the volatile portion of these by-products, and that the flavour of beer also is influenced by them. The results of experiments made by A. C. Chapman¹ to ascertain the nature and quantities of these bodies are given in the following table. They are expressed in percentages of the quantity of proof spirit present in each sample :

	A.	B.	C.	D.	E.	F.
*Higher alcohols (as amyl alcohol)	0.250	0.130	0.140	0.084	0.070	0.051
Compound ethers (as ethyl acetate)	0.036	0.044	0.052	0.037	0.024	0.021
Furfural	0.002	0.0006	0.002	0.0018	0.0006	trace
Aldehyde	trace	trace	heavy trace	heavy trace	trace	"

* Chiefly (A) propyl and amyl, (B) butyl, (C) amyl and butyl, (E) amyl.

A was a sample of XXXX beer sold in London; B, light bottled beer (carbonated); C, bitter beer; D, bottled beer; E, mild ale. Under F, are given the results of a similar examination of a sample of good Scotch whisky, which had been in cask for four years, for purposes of comparison.

The effect of a three months' storage of beer was to increase slightly the higher alcohols, the esters, and the furfural. The effect of very long storage is shown in the following table :

	A.	B.	C.
*Higher alcohols (as amyl alcohol)	0.140	0.102	0.110
Compound ethers (as ethyl acetate)	0.165	0.170	0.156
Furfural	0.0015	0.005	0.005
Aldehyde	trace	slight trace	slight trace
Proof spirit	20.0 p.c.	18.20 p.c.	19.50 p.c.
Fixed acidity (as lactic acid)	—	0.48 "	0.50 "
Volatile acidity (as acetic acid)	—	0.08 "	0.07 "

* Chiefly (A) amyl, (C) amyl.

A was a sample of Bass's beer which had been 20 years in bottle; B, a sample of beer with an original gravity of 1099; had been 16 years in bottle; C, this sample had been in bottle for over 20 years.

Antiseptics.—These substances, which are used as a sort of corrective for faulty materials or processes, are occasionally added to the mash liquor or to the contents of the mash-tun, a plan which, if not actually harmful, cannot be of any service. More generally they are added to the beer at the time of racking. All antiseptics have a prejudicial effect on the flavour of the beer, and some also, since they retard the action of the yeast during the secondary fermentation, on its conditioning. The antiseptics used in brewing

¹ *Journ. Fed. Inst. Brewing*, 3, 240.

are either the sulphites of calcium or sodium, or salicylic acid; the latter is but seldom employed at the present time (see below). Indeed so strong an antiseptic would only be required in the case of a beer actually diseased. Bisulphite of lime, and perhaps the so-called "potassium metabisulphite" (really anhydrosulphite $K_2SO_3SO_2$) are the most frequently employed antiseptics for preservative purposes; the former is also largely used for cleansing mains, tuns, and other plant. It is prepared by the manufacturers in a solution of a gravity of about sp. gr. 1072¹ degrees, and is added at the rate of from a quarter to a half pint to each thirty-six gallons of beer. Salicylic acid powerfully arrests the growth of disease ferments, but exerts a strong inhibitory action on the yeast also; it may considerably retard, or even completely arrest, the secondary fermentation; consequently, beers treated with this antiseptic are liable to drink flat and insipid. When employed, it is added at the rate of about a quarter of an ounce per barrel of thirty-six gallons. Formalin (an aqueous solution of formaldehyde) has been successfully used for the treatment of plant, but it should never be added to beer. Fluorides have been proposed as bactericides, and, undoubtedly, they are very powerful ones, but they should never be employed in brewing, because they are highly poisonous. Mixtures of sodium hypochlorite and caustic soda are now on the market, and are largely used as cleansing and sterilising agents for brewery plant, malting floors, &c. It is important that anything treated with these mixtures should be subsequently thoroughly swilled with water as their taste and odour are retained tenaciously.

Finings.—Beer fit for consumption should be absolutely bright and clear; in other words, all the matters which were in suspension should be either precipitated or removed. Owing to the taste now prevailing for an absolutely bright article, ales which do not fulfil these conditions are hardly saleable.

The tendency of all beers properly brewed from good materials is to drop bright spontaneously if stored for a sufficient length of time, but owing to the exigencies of the present day, in which quick consumption is the rule, sufficient time cannot generally be allowed for spontaneous clarification to take place; consequently it becomes necessary to employ some method which will rapidly remove suspended matters from the beer. Finings made by dissolving isinglass in sour beer or in a dilute solution of an acid are almost universally employed for this purpose.

Although other substances have been occasionally employed for the manufacture of finings, isinglass alone has been found to yield a really satisfactory product. Isinglass, the best qualities of which are made from the swimming-bladder of the sturgeon and other allied species,² comes into commerce in the form of thin shreds, leaves, pipes, and lumps; it varies in colour from white to deep yellow. The lighter coloured varieties command

¹ The value of bisulphite of lime when estimated from its specific gravity may be illusory; it should always be determined from the amount of sulphurous acid which it contains.

² On this subject an important paper by T. W. Bridge (*Journ. Inst. Brewing*, 1905, 11, 508) may be consulted.

the best prices, but the darker are said to yield the most powerful finings. The article should be perfectly sound and show no signs of decomposition, such as are indicated by an unpleasant odour and flavour. It should dissolve without difficulty in slightly acid solutions, and the less insoluble matter, or "skeg," as it is termed, which it leaves behind, the better.

Manufacture of Finings.—For this several large casks will be required; these are unheaded and provided with closely fitting covers; also two sieves, one coarse, the other fine. The quantity of isinglass required is about $2\frac{1}{2}$ lb. of the best qualities, or $3\frac{1}{2}$ lb. of the inferior ones, for each 36 gallons of finings. The amount of isinglass requisite for a batch, after having been weighed out, is placed in one of the casks, and as much water, to which the acid or acids to be employed have been added, poured in as will cover it. The cask is then closed, and the mixture well roused several times a day; enough water is added from day to day to keep the isinglass, which swells out considerably, submerged. When the "glass" is thoroughly softened, or, as it is termed, "cut," the mixture is passed through the coarser sieve, after which it is allowed to stand a few days longer. Finally, it is passed through the finer sieve, made up to the required bulk with water, and thoroughly roused, after which the finings are ready for use.

The Acids Used in Making Finings.—In former times sour beer was almost universally employed for the manufacture of finings, and it is considered by many that the most effective finings are obtained in this way. As sour beer is generally highly contaminated with acidifying bacteria, it is obviously a most unscientific procedure, and cannot be too strongly condemned. This objection, however, would not apply to sour beer which has been sterilised.

The acids which have been found most suitable for the manufacture of finings are acetic, tartaric, and sulphurous. The first of these cuts well, but yields an article which communicates an unpleasant flavour to the beer. Finings made with tartaric acid alone are apt to decompose and turn mouldy; hence, when this acid is employed, it is usual to add a small quantity of sulphurous acid, which acts as an antiseptic. These acids may be used in the proportion of 1 lb. of tartaric acid and 1 gallon of commercial (7.5 per cent. H_2SO_3) sulphurous acid for each 7 lb. of isinglass. It was supposed that acetic or tartaric acid cut better than sulphurous, but it has been shown by Matthews and Lott that this is not the case.¹ In a series of comparative experiments they found that sulphurous cut more quickly than the two former acids, which were themselves about equal in cutting power. The finings prepared with sulphurous acid were sound at the end of a month; those made with acetic acid were also sound, but somewhat mouldy; those made with tartaric acid were both mouldy and decomposed. It was found best to commence the cutting with a sulphurous acid solution of 1 per cent. strength, sufficient water being added during the process to reduce this down to 0.2 per cent. at the finish. These proportions are approximately obtained

¹ *Trans. Inst. Brewing*, 4, 201; see also Berry, *Journ. Inst. Brewing*, 1907, 13, 44.

by diluting one gallon of the ordinary commercial sulphurous acid (containing 7.5 per cent. H_2SO_3) with six gallons of water, and afterwards gradually adding water until the total quantity of finings amounts to 36 gallons. Lactic acid was also found to be a more effective cutting agent than acetic acid, and to give somewhat clearer finings than sulphurous acid. Lactic acid is the chief cutting agent when sour beer is employed.

Condition of the Isinglass in Finings.—Matthews and Lott consider that the “glass,” when converted into finings, is held by the acid in a state of true solution; this is, however, extremely doubtful, as the process of fining would appear to require a colloid. They were able to dilute the liquid portion of finings, strained off through a cloth, indefinitely, without any precipitate being thrown down. A dilute solution of finings could also be filtered through filter-paper. If, however, such a solution is made slightly alkaline, precipitation of the isinglass takes place.

Conditions of Fining.—The state of solution of the isinglass in finings is of a very unstable nature, and is affected by comparatively slight influences. Matters in suspension, such as cooler sludge or beer deposits, inert substances, such as powdered glass, asbestos, yeast in suspension, &c., were found to cause coagulation and precipitation of the isinglass. Beer turbidity, when caused by bacteria, cannot be removed by fining, nor will beers in a state of fret take finings. The peculiar haziness known as “greyness,” which is supposed to be due to the presence of nitrogenous matter in an extremely minute state of division, cannot be removed by fining. None of the ordinary sugars or dextrins appear to affect finings, but caramel causes precipitation, a large quantity of its colouring-matter being simultaneously carried down. Hop-resin in suspension also precipitates finings, and is readily removable by them. Alcohol appears to favour the action of finings. The presence of 0.4 per cent. of free acetic acid, in addition to the normal amount of acid contained in the beer (0.1 per cent.), does not prevent the action of finings, but rather accelerates it. As little as 0.2 per cent. of free lactic acid considerably impairs their efficiency, and it is questionable if beer containing 0.5 per cent. or more of this acid would take finings at all. Carbon dioxide under pressure can dissolve isinglass in water, but is unable to effect its re-solution when it has been precipitated in beer. Hop-tannin, which has always been supposed to be the principal agent in causing the coagulation of finings, is present in far too minute traces in beer to exert any action of consequence, though in the case of dry-hopped beers its action may be greater. Some brewers add catechu or cutch, which contains a large quantity of catechu-tannic acid, and this exerts a marked influence on fining. Fining, to be effective, must never be conducted at a temperature below 50° F.; and finings should never, either during their manufacture or afterwards, be heated, or they completely lose their efficiency. The action of finings is probably due largely to the phenomenon of “adsorption.”¹

¹ W. Biltz, *Ber. d. Deutsch. chem. Ges.*, 1904, **37**, 1095; J. L. des Banceis, *Comp. Rend. de l'Acad. des Sciences*, 1906, **143**, 174.

Methods of Using Finings.—Occasionally finings are added to beer in bulk, as, for instance, when it is in the settling or racking tank, but this plan cannot be recommended, although occasionally successful results have been obtained by it. The proper place to add finings to the beer is when it is in the trade casks, and then immediately before they are sent out. There are two distinct modes of fining, the one termed “fining in,” where the finings are permanently retained in the cask; the other, called “fining out,” in which the finings are ejected after a short time from the bung-hole of the cask. The chief merit of the latter mode, which is usually performed in the publican’s cellar, is its quickness of action. Beers treated in this way become, as a rule, brilliant in a few hours.

In “fining in,” the requisite quantity of finings is added to about three or four times its bulk of beer, the mixture well stirred or whisked together and poured into the cask, which is afterwards shived up and well rolled to distribute the finings. In “fining out,” the finings are similarly mixed with some of the beer, the whole returned to the cask, and then well stirred in with a stick. The bung-hole is left open, and in the course of a short time the finings are ejected, or, as it is termed, “sparged out.” The ejection is caused by the gas disengaged by the removal of pressure and by the agitation, which, becoming entangled with the coagulated finings, so lowers their specific gravity that they rise to the surface. In the former mode, no doubt, the finings float for a time from the same cause, but afterwards, as the entangled gas becomes absorbed owing to the increased pressure inside the cask, they sink.

Quantity of Finings Required.—For “fining in,” about one pint of good finings, containing the eighth of an ounce of isinglass per barrel, is, as a rule sufficient. Stubborn ales may require more, but the quantity used should not exceed two pints per barrel. Any excess beyond the quantity of finings absolutely necessary should be avoided, since they only increase the quantity of “bottoms” or precipitated insoluble matter in the cask. In “fining out,” a larger proportion may be used without any prejudicial result, since nearly the whole of the added finings are ejected.

Bottling.—Ales produced for bottling are of two entirely distinct types. To the former belong the older varieties of strong, bitter, and pale ales, which are allowed a considerable length of storage in cask previous to being bottled. To the latter belong the lighter ales of more modern origin, in which the storage is shortened to periods varying from six to two months according as a more or less ripe and mature article is demanded in the locality in which they are consumed. It includes also the very light bottled beers which have come so much into vogue lately, and also those in which the cellaring previous to, and storage after, bottling are reduced to a minimum, condition being brought about artificially by impregnation with carbon dioxide gas; also those which in addition to being carbonated are chilled and filtered.

Strong Bottled Ales.—In the production of beers of this class, only the best materials are employed, and these are brewed in the most careful manner

with water containing calcium sulphate in considerable amount. They are often produced from malt alone, but more usually with 25 per cent. of glucose, and occasionally a small proportion of unmalted material as well. Hops are used at the rate of from 14 to 15 lb. per quarter. The ale undergoes a protracted period of storage in cask, so that secondary fermentation is almost completed. Ales of this class should drop bright spontaneously. Immediately before being bottled any excess of gas is allowed to escape by means of porous spiling, care being taken that the beer is not flattened too much or it may absorb oxygen from the air, and this would be fatal to its subsequent brilliancy in bottle. The beer is run into the bottles by means of a tap, the nozzle of which is long enough to reach to the bottom of the bottle, or by means of a bottling machine. The bottles are corked immediately they are filled, and, as recommended by Pasteur, placed on their sides for twelve or twenty-four hours, in order that the oxygen in the small amount of included air may be absorbed, after which they are stood upright in the store cellar, which should be maintained at an even temperature of about 55° F. Just before consumption they are stored for a week or a fortnight at a temperature about 10° higher in order to bring them into condition. All bottles employed for bottling ales should be cleansed with the greatest care, and afterwards thoroughly dried, best by means of hot air; moisture left in the bottles simply acts as a trap for germs.

Light Bottled Beers.—In the production of these a blend is used consisting of good sound English and foreign malts, invert sugar or glucose. In some cases a small proportion of prepared material, such as flaked maize or rice, is used in addition. A. C. Chapman¹ recommends the employment of some form of limited decoction process of mashing, such as that described on p. 518, in conjunction with a brewing liquor containing a fair amount of calcium sulphate and alkali chlorides. In this way considerable body and palate-fulness are said to be secured. Hops may be used at the rate of 6 to 8 lb. per quarter of malt. The period of storage in cask for this class of beers varies in different localities from six months to a few weeks; where short periods of storage are adopted the secondary fermentation has to be hurried along by frequent cask-rolling, the porous spile being frequently used, and great care is taken that the cask changes do not proceed too far before the beer is bottled off. The ale under these circumstances rarely drops bright of itself; consequently fining has to be resorted to, and the smallest quantity of finings which will effect the object in view is used. The finings are added to the beer, previously freed from any excess of gas, a week or a fortnight before bottling takes place. It is then bottled off under all the precautions described above in connection with the bottling of strong ales, after which the beer is stored in a room kept at a constant temperature of about 65° F., so as to bring it rapidly into condition.

Heating of Conditioning-Rooms for Bottled Beers.—The store-rooms for this purpose are often heated during the colder months of the year with coke

¹ *Journ. Fed. Inst. Brewing*, 1896, 2, 274.

fires contained in braziers, but this is a very rough and ready way, since the heat is never equally distributed throughout the room. A much better plan, suggested by Mr. Fletcher, is to instal a number of ordinary gas jets at the level of the floor, which have their supply of gas regulated by one tap on the main. This will need adjusting several times in the twenty-four hours, so that the temperature, as indicated by a thermometer suspended in the room, may be kept as equable as possible. Larger storerooms may be economically and efficiently heated by means of four-inch cast-iron pipes, into which are inserted at intervals small steam jets, fed from the steam-boiler and regulated by one valve on the steam main. The large pipes are conveniently sunk under the floor and covered with gratings. A steam-trap, or some other means of getting rid of the condensed water, is a necessary adjunct. A small boiler, heated by gas, in connection with a coil of pipes, arranged in the same way as in a greenhouse, also forms an economical and efficient apparatus for keeping up the temperature of small conditioning-rooms.

Carbonated Bottled Beers.—As beers of this class are intended for rapid consumption, the materials used in their production need not be of the choice quality necessary in the two former cases. The mashing liquor should contain a smaller quantity of calcium sulphate, and a fairly large amount of the alkali chlorides, and the form of limited decoction method of mashing just mentioned may be advantageously employed. The beer is all the better for being stored for six weeks or two months before being bottled; after storage it is fined, and, as soon as bright, carbonated and bottled off in one of the numerous machines constructed for this purpose, when it is ready for immediate consumption. It must be remembered that the flavour due to carbon dioxide in artificially carbonated beers is at first distinctly different from that of those in which the gas is generated by slow fermentation in bottle, but if such beers be kept for a time the flavour improves. In the former case, the gas may be perhaps, to a great extent, merely in a state of solution; whilst in the latter it may be combined with water and exist as carbonic acid, which is formed in the following manner: $\text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3$. Probably, as suggested by Professor Liebrich, traces of ethyl carbonate are formed during the secondary fermentation, and this ether confers its agreeable flavour on beers of the latter class.

Non-Deposit Beers.—When an ordinary top fermentation beer is cooled to a temperature near the freezing-point of water, it becomes turbid owing to the separation of various albuminoid substances and hop resins; but these for the most part redissolve when its temperature is again raised, the beer thus as a rule remaining hazy. If beer, which has become turbid by cooling, is filtered bright it retains its brilliancy unless cooled to a temperature below that to which it has been subjected previous to filtration. Advantage was probably first taken of these facts in the United States of America, where during the summer there is great demand for iced beverages. At the present time there are, broadly speaking, two systems of producing non-deposit bottled ales. The first is the slow system, in which the beer is allowed to get into

condition either naturally by storage in cask or is forced into condition by the addition of fermentable sugars as priming, or of "kräusen" (fermenting wort). In either case specially strong casks or metal tanks are used. The requisite condition having been attained, the beer is chilled to from 26–38° F., filtered, and bottled. In this system the beer is got into high condition in cask, and the plant is so constructed as to prevent any loss of gas during the subsequent treatment. In a modification of the system, the beer after storage for a week or so, during which it may or may not reach high condition, is chilled, and before filtration carbonated by being sprayed under pressure through an atmosphere of gas collected from the fermenting tuns. The second system of producing non-deposit ales is the so-called rapid system in which the beer is passed direct from the settling back into tanks or cylinders in which it is chilled by appropriate means and having been carbonated, subsequently filtered and bottled. For further information the reader is referred to the papers by H. T. Brown,¹ H. Van Laer,² Norbert Van Laer,³ and C. H. Field,⁴ P. K. Le May,⁵ P. Dreesbach⁶ and A. Hartley.⁷

¹ *Journ. Fed. Inst. Brewing*, 1897, **3**, 467.

² *Ibid.* 1898, **4**, 24.

³ *Ibid.* 1900, **6**, 438 : 1903, **9**, 63.

⁴ *Journ. Inst. Brewing*, 1905, **11**, 107.

⁵ *Ibid.* 1907, **13**, 2.

⁶ *Beer Bottlers' Handy Book*, Chicago, Wahl-Henius Institute, 1906.

⁷ The Bottling of English Beers, London. *The Brewing Trade Review*, 1906.

CHAPTER X

BEER, ITS CHARACTERISTICS AND ITS DISEASES

BEER fit for consumption should have an agreeable flavour and aroma, be well charged with carbonic acid gas, should possess the requisite palateness according to its class; further, when being poured into a glass, it should form a persistent foamy head. In accordance with the present taste, it is absolutely essential that beer should be perfectly clear and brilliant. The acidity should be so slight as to be hardly perceptible.

The flavour and aroma depend materially upon the employment of good sound materials, for it is obviously impossible to brew a good-flavoured beer from fusty malt, and it is equally impossible to secure the proper malty flavour from raw and under-cured material. Manipulation has also much to do with flavour, so also has the quality of the water employed. The aroma will depend materially upon the quality of the hops used; it cannot be expected that an article with a fine aroma will be produced if only coarse-flavoured hops are employed in its manufacture.

The species of yeast employed has, as was first pointed out by Hansen, much influence on the flavour of beer, each different species, and indeed variety, possessing different properties in this respect. Those who advocate the employment of yeast derived from one single cell, claim that inasmuch as one variety only is thereby made use of, one uniform flavour is secured in the finished beer. It was first pointed out by Hansen that the yeast which he had isolated and named *S. Pastorianus No. I.* was able to develop an intensely nauseous flavour in beer. Grönlund states that *S. Pastorianus No. II.* is also able to communicate a bitter twang to beer, and various other yeasts possessing similar properties have since been discovered by other observers. Another yeast, Hansen's *S. anomalus*, readily ferments wort, and communicates to the beer a fruity flavour.

The *Torula*, which are frequently met with in wort, do not, as a rule, cause disease in beer; one species which Grönlund met with, and named *T. nova Carlsbergiae*, ferments maltose, cane sugar, and glucose, and develops in beer an unpleasant bitter taste. Lafar has also isolated a mycoderma-like fungus from beer which produces acetic acid.

The bacteria met with in wort and beer principally cause trouble by the development of acidity. Those which have been the longest known are the

lactic ferments described on p. 273. They are all rod-shaped organisms, with the exception of Lindner's *Pediococcus acidi lactici*. The butyric ferments (p. 272) are rarely met with in beer. When they do intrude they communicate to it the intensely nauseous odour and flavour of butyric acid. Acetic bacteria occasionally infect beer, and when permitted to develop there, give it a vinegar-like flavour, and turn it sour from the production of acetic acid. As these organisms cannot thrive without air, they are only met with in beer which has been allowed to become flat, and to which air has been allowed access. As a producer of bad flavour may also be mentioned the *Pediococcus cerevisiæ*, described by Balleke,¹ and a similar organism found by Reichard,² and termed *P. sarcinæformis*. It produces chiefly alcohol and lactic acid.

Condition.—This is maintained (except where the beer is artificially impregnated with gas) by the constant slow evolution of carbon dioxide during the secondary fermentation, which is secured by leaving a certain amount of the slowly fermentable maltodextrins in the beer at the close of the primary fermentation. The nature and quantity of these will depend materially upon the particular class of beer; ales which are stored for long periods will require fairly large quantities of very slowly fermentable bodies, those for quicker consumption less of these and more of the readier fermentable bodies, which should be exactly fitted to the type of the beer. If, in the case of store beers, too much readily fermentable matter is left, fretting ensues and in the end too much extract is fermented away; such beers consequently, at the time they should be ripe and fit for consumption, drink thin and poor. In beers for quicker consumption, if these matters are too large in quantity or of too fermentable a nature, a tumultuous secondary fermentation is induced. This keeps the yeast in suspension, and, as a consequence, such beers will not take finings. Should, on the other hand, these slowly fermentable bodies be present in too small a quantity, then the secondary fermentation either entirely ceases or is so small that the beer is not kept in condition. It becomes flat in the cask, and in this state is especially amenable to the attacks of any disease ferments which it may happen to contain.

Palate-Fulness.—The amount of palate-fulness required in a beer will depend materially on its type, and on the taste prevalent in the neighbourhood where it is consumed.

Beers should never taste thin; an amount of extract should always be left after fermentation sufficient to give the necessary body. As to the nature of those constituents of the extract which essentially contribute to palate-fulness, little is known with certainty. This property does not appear to be altogether dependent on the quantity of the extract left, but rather on its quality; for of two beers brewed at the same gravity and fermented to the same degree of attenuation, the one may drink full and round, the other thin and poor. The more highly coloured malts, that is, those which have been exposed to a greater degree of heat on the kiln while containing a fairly high

¹ *Woch. f. Bran.*, 1884, p. 185.

² *Zeit. f. g. Bran.*, 1894, p. 257.

percentage of moisture, produce fuller beers than the pale varieties. Constituents which have a sweet taste also confer palate-fulness on beer, and this is heightened by the simultaneous presence of sodium chloride. Some years ago H. T. Brown suggested that the fulness of beers brewed from waters containing chloride might perhaps be ascribed to the fact that chlorides dissolve the globulins of the malt. The effect of priming in giving fulness is well known; it probably depends to some extent on the sweetness of the sugar used, and also on the increased amount of carbon dioxide evolved, for beers well charged with gas drink much fuller than flat samples. It is extremely probable that the nitrogenous constituents help in this direction, as also do the gummy and pectinous bodies of the malt. Prior¹ is inclined to attribute some of the palate-fulness of beer to the kind of yeast employed; thus it is often found, in two beers produced from the same materials and brewed on exactly the same lines, that the one fermented with one variety of yeast will be much fuller on the palate than that fermented with another variety. This he attributes to the gummy substance which some yeasts secrete in much greater abundance than others.

Thinness in Flavour.—This may be brought about by too great a reduction of the extract during the primary fermentation. Even when a sufficient amount of extract has been left, and this of the right quality, it may be removed by the action of the yeasts during the secondary fermentation, should these be of such a nature as to be able to hydrolyse and ferment the higher maltodextrins. Many bacteria also secrete diastatic enzymes, which, acting on the higher maltodextrins, render them amenable to the yeast. There seems to be no definite relation between the viscosity of a beer, as ascertained experimentally, and its palate-fulness. Beers which contain more than the normal amount of acidity also taste thin.

Head.—Good beer should, upon being poured out into a glass, form a good foamy head, capable of persisting for some time. The formation of this head depends upon two factors, the presence in the beer of a sufficient quantity of combined carbonic acid and of bodies which give it a certain amount of viscosity. This enables the beer to entangle and retain the liberated bubbles of carbon dioxide as a foam on its surface. What the particular constituents of the extract are which confer this property on beer is not known with certainty; it is supposed to be dependent on the gummy and pectinous substances of the malt, and perhaps to some extent to the higher maltodextrins and to the hop-resin. Wahl and also Windisch² attribute viscosity to the proteoses; these, when separated by salting out either with ammonium or zinc sulphate and redissolved in water, give a solution which readily forms a persistent foamy head. The latter considers that a sufficient amount of the proteoses is already formed in the malt, and that any further proteolytic action on them in the mash tun, such as that which can be brought about at temperatures under 150° F., is undesirable.

¹ *Chem. u. Physiol. d. Bier*, p. 502.

² *Woch.f. Brau.*, 1893, p. 1358; 1896, p. 1253; 1897, p. 21.

Brightness.—As already observed beer ought to be perfectly clear and brilliant; it should show no sign of opalescence, dimness, or turbidity. The causes of a reverse condition of things are manifold. A more or less turbid condition may be caused by unorganised suspended matters, such as starch, dextrin, proteïds, galactoxylan, or hop-resin. Turbidity from starch and dextrin are rare, and can only arise from the employment of an improperly modified malt of low diastatic power, or from some fault in the mashing process by which too much diastase is destroyed, or through the use of raw material in too large a quantity. Turbidity from this cause can be removed by the addition of cold-water malt extract. The proteïds also at times develop turbidity; the cause for this may be, according to Prior,¹ under-germinated and incompletely modified malt; material made from barley too rich in nitrogen; too small an increase of yeast during the fermentation; or too low a degree of attenuation. The slightly soluble compounds which the proteïds of the malt form with the tannin of the hops are probably the cause of that form of turbidity which appears in beers when their temperature is reduced, and which disappears as the temperature again rises. A rare form of haziness is said to be caused by the hop-resins being partially thrown out of solution; it probably never arises when good sound hops are employed, and is probably due to some prejudicial change having taken place in the hop-resins either during the harvesting or storage of the hops.

One of Hansen's most remarkable discoveries is that beer turbidity can be caused by certain species of yeast; this he first showed to be the case with reference to *S. Pastorianus* I. and III. and *S. ellipsoideus* II., and several other yeasts have been found which behave in a similar manner. But it has been decided by direct experiment that these "wild yeasts" can only do harm when they are present beyond a certain quantity; when below this they are held in check by the ordinary yeast. It has also been shown by Hansen that two yeasts, which individually give bright beers, may, when employed in combination, yield a turbid beer; this was found to be the case with the *S. cerevisiæ* Nos. I. and II. of the Carlsberg Brewery.

It must be remembered that yeast turbidity is a normal condition during the primary fermentation, and it only becomes an abnormal one when prolonged beyond a certain stage. There are considerable differences in the way in which the various cultivated yeasts behave at the close of the primary fermentation; some have a tendency to clot together in small aggregates and leave the beer clear, others appear to be of a more pulverulent nature, and there are still others which remain in suspension for a considerable time. The brewer who requires quick clarification will avoid the latter. Slow brightening, however, does not always denote a bad quality of yeast; for instance, the beer fermented with the Carlsberg *S. cerevisiæ* No. I. takes much longer to clarify than the beer brewed with No. II., but it is more stable. Yeast turbidity, unless the beer is in a state of fret, is removable by fining.

¹ *Chem. u. Physiol. d. Bier*, p. 508.

Another set of organisms nearly allied to the *Saccharomycetes*, the *S. mycodermae*, are frequently found as a film on beer (see pp. 264, 265). Lasche¹ has described four varieties of these organisms, of which Nos. 1, 3, and 4 were able to cause beer turbidity, while No. 2 did not appear to act in this way. The most serious kind of beer turbidity, however, is that induced by bacterial organisms, for when due to this cause it cannot be removed by finings, and it is generally associated with such profound alterations in the odour and the flavour of the beer as to render it unsaleable. All the bacteria which have been mentioned in connection with flavour, with the exception of Pasteur's lactic ferment, produce turbidity.

It has been a much-debated question as to whether the *Sarcinae* are able to cause disease in beer or not. Balcke² found that the *P. cerevisia* caused turbidity and communicated an unpleasant flavour, and this was confirmed by Reincke³ as well as by Lindner. On the other hand, Petersen and Hansen⁴ regard this organism as a perfectly harmless one. Probably different species of *Sarcinae* exist so much alike in appearance that it is impossible to differentiate them microscopically, some of which are innocuous, others not. The *P. sarcinaeformis* of Reichard is often present in wort or beer without causing trouble; at other times it evinces an intense virulence. This observer showed that the noxiousness of the organism was much influenced by the temperature at which it was cultivated, low temperatures increasing, high temperatures diminishing its virulence. When pediococci, which had been cultivated in unhopped wort, were added to wort before the primary fermentation, they did not manifest signs of malignancy; but when the organisms had passed through a fermentation they became virulent, and those which were derived from an infected beer were the most baneful. The organism showed its influence to the greatest extent in weakly attenuated beers, in which the secondary fermentation had been violent. When growing in an anaërobic condition amongst the sedimentary yeast at the bottom of the cask, it appears to be harmless, but when, as in a fretty beer, it is brought to the surface by the rapidly evolved gas, it there meets with small amounts of oxygen, and these appear to increase both its powers of reproduction and its virulence.

Ropiness.—This condition may be induced by the *B. viscosus* Nos. I. and II., described on p. 272. It is only when these organisms obtain access to the wort before the primary fermentation that they are able to exert their baneful influence; when added afterwards, they do not appear to exert an injurious action. According to Lindner, beers containing much assimilable nitrogenous matter, and also beers which have been attenuated to a considerable extent, are the most liable to be affected in this way. The ropiness is caused by the production of two mucilaginous substances by the bacteria, one of which contains nitrogen and is insoluble, the other is nitrogen-free and soluble in water. When the wort contains an amount of acid equal to 0.125 per cent. lactic acid, these organisms do not seem able to cause ropiness.

¹ *Der Braumeister*, 1891, p. 200.

² *Woch. f. Brau.*, 1884, p. 105.

³ *Ibid.* 1885, p. 748.

⁴ *Zeit. f. Brau.*, 1890, pp. 1 and 7.

The *Pediococcus viscosus* (page 274) has been found to cause ropiness in German white beer. A series of intermittent outbreaks of this disease in beer was traced by H. T. Brown and Morris¹ to the aërial infection of the worts when on the cooler by this or some other closely allied organism. It formed small yellow, wax-like glistening colonies on meat-broth gelatin. Its habitat was traced to the soil of a yard adjoining the fermenting room, occupied by a pork-butcher, which was polluted with putrid animal refuse.

As certain bacteria are such dangerous foes, it should be one of the brewer's first cares, by the strictest attention to cleanliness in every portion of the brewery itself, in the plant, and especially in the wort mains, to eradicate as far as possible these obnoxious organisms. Even with all possible care they cannot be absolutely exterminated; yet, if kept in subjection, they are unable to do harm; it is only when allowed to get the upper hand that they are able to evince their mischievous propensities. Thanks to the teachings of Pasteur, their depredations are not now nearly so frequent as they formerly were; it is as rare nowadays to meet with samples of yeast swarming with bacterial organisms as it was common twenty years ago.

¹ *Journ. Fed. Inst. Brewing*, 1895, 1, 14.

APPENDIX

Starch.—Researches of Maquenne and Roux.¹ (Compare p. 75).—Following Nägeli and others, natural starches are generally regarded as a mixture of at least two substances, granulose and amylocellulose or starch cellulose, the chief portion consisting of the former, and the latter being present to the extent of 3–4 per cent. According to this view amylocellulose is in a sense to be regarded as extraneous to the actual constituents of the starch granules. Maquenne and Roux, on the other hand, have recently brought forward evidence that amylocellulose in different states of condensation forms the principal constituent of the granules, their latest view being that 80–85 per cent. of the natural starches consists of that substance in admixture with another substance, to which they give the name amylopectin; it is asserted that the viscosity of ordinary starch-paste is due to the presence of this latter substance which has, however, never been isolated. It is dissolved (liquefied) by diastase, but is only very slowly saccharified by that enzyme.

Amylocellulose separates when starch-paste or starch solution is allowed to stand; the amount deposited increases with the duration of the stand, and the moderate addition of acids augments the amount, whilst alkalies also produce an increase at first; subsequently, however, they cause a diminution by dissolving it. Fernbach and Wolff (see p. 75) have shown that this reversion of starch is promoted by amylocoagulose. By the aid of this enzyme as much amylocellulose may be obtained in half an hour as is produced by allowing a solution of starch to stand in the cold for a week.

Amylopectin.—Up to the present time it has not been possible to isolate this substance, the existence of which is therefore purely hypothetical. In the first place starch-paste cannot be filtered, nor can the gelatinising principle be separated by centrifuging. Further all reagents, known to act upon it, liquefy it and thus convert it into a form in which it cannot be separated from the other constituents.

Artificial Starch Granules. *Amylose.*—Maquenne and Roux prepare amylocellulose by keeping a starch-paste at a low temperature for a week. The substance which deposits is collected and washed; it is then heated with water at 120° C. and each time submitted to the action of malt extract at

¹ *Annales de chim. et phys.*, 1904, [8], 2, 109–135, 1906; [8], 9, 179–220.

56° C. until the iodine reaction disappears. The residual substance is again collected, washed and dried over sulphuric acid in a vacuum, when it is obtained as a hard granular mass containing about 1.5 per cent. of siliceous ash and a little nitrogenous matter derived from the malt extract. It is practically insoluble in water at 120° C., but dissolves at about 150–155° C., when the temperature is raised by degrees. When a solution obtained by heating for 10 minutes, as just described, is allowed to cool, it becomes viscous, opalescent, and finally the whole is converted into a gelatinous mass which gives a blue coloration with iodine. If, on the other hand, the heating be prolonged for an hour or more a perfectly clear solution is obtained, which slowly deposits granules resembling natural rice starch. These artificial starch granules are said to consist of pure amylocellulose or, as Maquenne and Roux prefer to term it, *Amylose*. They consider that this substance exists in the natural starches of which it forms the chief portion. As evidence that it is not produced during the process of heating the starch with water at 150° C., they point out that when amylose is so heated a less soluble substance is never obtained; that, on the contrary, prolonged heating causes hydrolysis into dextrins and sugars, from which it is impossible to regenerate the original substance. The artificial starch granules differ from natural starch granules in not giving a paste with boiling water, and by dissolving in alkalies without previously swelling.

The amyloses of natural starch are completely soluble in boiling water, whilst those composing artificial starch granules are insoluble and give no coloration with iodine unless kept in a humid atmosphere for some time. Maquenne and Roux explain this difference by the fact that the amyloses of the artificial granules are in a more highly condensed state than those of the natural starches which contain these same amyloses mixed with their lower homologues; these latter are much more soluble and play the rôle of solvents towards the higher members.

Maquenne and Roux have compared the action of malt extract at 50° C. on starch-paste and on a solution of so-called pure amylose. Their results are shown in the following table:

Time.	Percentage of Maltose on original Starch Substance.	
	Starch Paste.	Amylose.
5 minutes	66.7 per cent.	94.4 per cent.
15 "	74.9 "	98.1 "
30 "	76.9 "	99.7 "
45 "	— "	99.6 "
1 hour	79.0 "	99.7 "
1.5 hours	— "	100.0 "
2 "	81.1 "	100.1 "
2.5 "	— "	100.0 "
3 "	82.0 "	101.4 "
28 "	91.8 "	104.2 "

They consider that the liquefying and saccharifying actions of diastase are due to two distinct enzymes; namely, one which converts amylose into maltose, which may be called amylase; and another which converts

amylpectin into maltose which, following Wijsmann and Duclaux, may be called dextrinase. The fact first pointed out by Brown and Morris that there is an apparent halt in the reaction in the case of starch-paste when about 80 per cent. of maltose is formed is explained by Maquenne and Roux on the assumption that the hydrolysis of the amylose is then complete. In further support of the view that starch consists of about one-fifth of amylopectin, Maquenne and Roux point out that the coloration produced by the same quantity of iodine on a solution of amylose is about one quarter more intense than on a solution of starch of the same concentration, both solutions being prepared at 150° C.

Auto-excitation of Malt Extract.—Maquenne and Roux state that the activity of malt extract towards starch solution or starch-paste, but not towards solutions of amylose, is increased on allowing it to stand. The phenomenon which they denote by the term auto-excitation is, according to them, connected with the proteolysis of the protein compounds present in the malt, and, adopting for the purpose of argument the hypothesis of Maquenne and Roux as to the nature of diastase, the excitation is confined to the dextrinase. Malt extract was kept for a week and a month, at the end of which periods 100 and 103 per cent. respectively of maltose were obtained by its action on starch-paste at 50° C. in 24 hours, whereas the same extract when freshly prepared gave only 94 per cent. of maltose *ceteris paribus*. This increased activity is observed when the malt extract is kept at both high and low temperatures, provided the limiting temperature of diastatic activity be not exceeded. Even at a temperature as low as 56° C., however, the initial increase of activity is counterbalanced after some hours by a weakening due to the effect of heat. An excitation of malt extract is produced instantaneously by the addition of a certain quantity of a strong acid. This fact was first pointed out by Kjeldahl, who gave no indication, however, of the amount of acid necessary. Maquenne and Roux state that to obtain the maximum amount of maltose from starch-paste, which is always alkaline, it must be made neutral to Poirrier's orange No. 3; the malt extract must also be neutralised to the extent of one-third or two-fifths, according to circumstances (see Ford, p. 184).

As evidence that starch-paste may be hydrolysed by malt extract to a greater degree than is usually stated in the literature, Maquenne and Roux quote Effront, who obtained 96 per cent of maltose in three or four days by acting on starch with malt extract in presence of ammonium fluoride; they obtained the same result by substituting toluene for ammonium fluoride. The writer may here point out that Ling and Davis have shown¹ that starch-paste is hydrolysed in 42 hours at 55° C. by an active preparation of diastase to a point at which the constants² (specific rotatory power and cupric reducing power) are substantially those of maltose, and that in such a solution these

¹ *Journal Fed. Inst. Brewing*, 1902, 8, 482.

² Ling and Davis's constants are calculated on the total solid matter in solution determined from the specific gravity by the factor 3.93, and not on the original starch,

observers detected nothing but maltose. One of their experiments may be quoted here.

Diastase from a sample of low-dried Odessa malt was allowed to act on potato starch paste at 55° C. The following results were obtained :—

Time.	[α] D _{3.93}	R _{3.93}
1½ hours	150.0°	78.8
18½ „	143.6°	90.6
42 „	138.5°	99.7

This work of Ling and Davis is here mentioned as showing that apart from any hypotheses as to its nature, the fact that starch could be converted completely into maltose by diastase on the evidence of the reducing and optica rotatory constants of the final product was known before the researches of Maquenne and Roux were published. It is open to doubt, however, whether the final products obtained by Ling and Davis at 55° C., and by Maquenne and Roux at 50° C., consist entirely of maltose, although the dissolved matter may have the constants of that sugar (compare Ling, pp. 182–183).

In the writer's opinion the evidence brought forward by Maquenne and Roux in support of their views on the constitution of natural starches and on the mechanism of diastatic hydrolysis is far from conclusive, and will need supplementing to a large extent before their views can be accepted.

The Nitrogenous Constituents of Malt which are Soluble in Cold Water and are not Coagulated on Boiling.¹ (Compare p. 193.)—Since the proportion of soluble nitrogenous matter to non-nitrogenous matter is much higher in malt coombs than it is in malt itself, the aqueous extract of the former was first investigated. From the extract asparagine, allantoin, betaïne, and choline were isolated. The uncoagulable nitrogenous constituents of malt were then examined, a cold water extract being employed in the first instance. The cold water extract was boiled and filtered in order to remove coagulable nitrogenous matter, and then concentrated *in vacuo* to a specific gravity of 1.155.43.

Of the nitrogen present in a boiled cold water malt extract, about 51 per cent. is precipitable under certain conditions by phosphotungstic acid; the remaining 49 per cent. can be referred to (a) nitrogen in the form of ammonia, determined by distillation *in vacuo* with magnesia; (b) nitrogen as acid amides (*e.g.*, asparagine, &c.) liberated as ammonia on acid hydrolysis; (c) nitrogen provisionally referred to monamino acids (*e.g.*, aspartic acid, leucine, tyrosine), determined by liberating the nitrogen with nitrous acid. Assuming that the last reaction gives a measure of the amino acid nitrogen, about 70 per cent. of the total nitrogen of the phosphotungstic filtrate remains unaccounted for; this is provisionally referred to “unclassified nitrogenous substances.”

On the assumption that these unclassified bodies yield no ammonia under

¹ H. T. Brown, F. Escombe, A. McMullen, and J. H. Millar (*Trans. Guinness Research Laboratory*, 1906, 1, Part ii., 166–283).

the limited hydrolytic process to which the solution is subjected in estimating the amides, and that they do not yield any free nitrogen when treated with nascent nitrous acid, the analytical results obtained as above, referred to the original boiled cold water extract, are as follows :—

	Per cent. of total nitrogen.
Nitrogen precipitable by phosphotungstic acid	51.00
Nitrogen left in the phosphotungstic filtrate—	
Present as ammonia	1.34
,, amides	3.44
,, monamino acids	9.15
,, unclassified nitrogen	35.07
	<hr/> 100.00

Subsequent investigations have shown that free nitrogen is liberated from the “unclassified” bodies by nitrous acid, hence the monamino acid estimate is too high. In dealing with a complex mixture of nitrogenous substances such as occurs in malt extracts, it was desirable to find some property by which the substances could be characterised at any stage of their separation, so that a comparison of the mean properties of the fractions and of the original mixture would render it possible to judge whether any changes of fundamental importance were brought about by the methods of separation. Some criterion was required similar to the cupric reduction or optical activity. The behaviour of the substances towards nitrous acid furnished a provisional method of the kind. The ratio of half the amount of nitrogen liberated by nitrous acid to total nitrogen (by Kjeldahl), multiplied by 100, gives the apparent percentage of nitrogen in the form of amino acid. This ratio is referred to as the amino index and is denoted by the symbol “AoN.” Both precipitate and filtrate from the phosphotungstic acid treatment liberate nitrogen on treatment with nitrous acid. The organic bases are present for the most part in the precipitate, the amides and amino acids chiefly in the filtrate. The “unclassified” bodies are found both in precipitate and filtrate. In investigating the “unclassified” nitrogen, it was found preferable first to extract the malt with 70 per cent. alcohol. The alcoholic solution, on evaporation *in vacuo* and exhaustion of the residue with hot water, gave an aqueous solution which is referred to as the “alcohol-water extract”; it contains all the organic bases, amides and amino acids of the malt, and a considerable amount of the “unclassified” bodies. The latter can thus be obtained, by suitable means, in a more concentrated form than when using the original aqueous extract. The extraction with water of the malt residue after treatment with alcohol, gives the balance of the water-soluble uncoagulable nitrogen and of the soluble carbohydrates. From the phosphotungstic filtrate of the “alcohol-water extract,” asparagine, tyrosine, leucine, and allantoin were separated; from the precipitate itself, betaine and choline were separated; the greater part of the precipitate, however, consisted of “unclassified” bodies of the same nature as those occurring in the filtrate. The “unclassified” nitrogenous bodies consisted chiefly of what were termed

malt albumoses (salted out by ammonium sulphate and in much larger quantity by zinc sulphate) and malt peptones. The malt albumoses, which can be salted out of the alcohol-water extract by ammonium sulphate, have an amino index of about $AoN = 5$, and belong to a special class of these bodies, since they do not give the biuret reaction. The aqueous solution, even when very dilute, gives a persistent froth on shaking. These albumoses may be differentiated with 85-per-cent. alcohol into malt albumose I ($AoN =$ about 4.0), and malt albumoses II ($AoN =$ about 5.0); the former only gives the persistent froth in aqueous solution. The malt peptones, provisionally so called, left in solution after salting out, resemble, in many respects, the ordinary peptones of animal origin, but do not give the biuret reaction, and are not precipitable by iron-ammonium alum. From the investigation of the "alcohol-water" extract, and from the estimation of the ammonia by the magnesia method, and that of the albumoses by salting out, the following distribution of nitrogen in the cold-water extract of malt has been deduced :—

Ammonia nitrogen	3.5
Malt albumose nitrogen	20.0
Malt peptone nitrogen	31.0
Amide and amino-nitrogen	8.5
Nitrogen due to organic bases	4.0
Balance of nitrogen unaccounted for	33.0
	<hr/>
	100.0

The missing nitrogen of the cold-water extract is found in the malt residue after complete extraction with 70 per cent. alcohol, from which it can be extracted by suitable treatment with water. According to Osborne, it appears that the alcohol-soluble protein of barley is the one which suffers the largest amount of change into water-soluble uncoagulable bodies during malting. By the Hausmann method of nitrogen division, the malt albumoses and malt peptones isolated are shown in all probability to be a series of transformation products of this alcohol-soluble protein, as the amide nitrogen shows a progressive decrease and the basic nitrogen a complementary increase. The fact that a body, very similar to one of the malt albumoses, can be produced from the alcohol-soluble protein by partial hydrolysis, supports the last conclusion. This hydrolysis is characterised by the partial elimination of the amide and amino constituents of the protein molecule, whereas the basic portion persists in the final product isolated.

Evidence is brought forward to show that the malt albumoses and malt peptones may be regarded as a series of albumoses and peptones whose departure from the conventional ideas of these bodies is determined solely by the type of protein from which they are derived.

Migration of Nitrogen from the Endosperm to the Embryo during the Limited Germination Occurring during the Malting Process.¹ (Compare p.

¹ H. T. Brown, F. Escombe, A. McMullen, and J. H. Millar (*Trans. Guinness Research Laboratory*, 1906, 1, Part ii., 284-287).

302).—When a barley embryo is excised and grown apart from the endosperm in water culture, development soon ceases owing to lack of nutriment. If carbohydrate food is supplied in the form of cane sugar, growth is more prolonged but ultimately ceases owing to nitrogen-hunger; under these conditions the root development is somewhat feeble, and the tissues are rather flaccid. Hence, it would appear that with a grain of barley growing under natural conditions, nitrogen is transferred from the endosperm to the embryo even in the earliest stages of germination. That such a transference does occur has already been demonstrated. The migration of nitrogen from endosperm to embryo during intervals of two days on the malting-floors has now been investigated. The barleys employed were (1) a Hungarian Chevallier, and (2) an Irish Chevallier. The following results were obtained :

	I. Hungarian barley.		II. Irish Chevallier.	
	Percentage of nitrogen left in endosperms.	Percentage of endosperm nitrogen which has migrated to embryos.	Percentage of nitrogen left in endosperms.	Percentage of endosperm nitrogen which has migrated to embryos.
After steeping . . .	100.0	—	100.0	—
3rd day on floor . . .	91.1	8.9	86.2	13.8
5th " " . . .	82.8	17.2	83.0	17.0
7th " " . . .	71.2	28.8	82.8	17.2
9th " " . . .	64.6	35.4	67.8	32.2
1st day on kiln . . .	65.5	34.5	—	—

These results show that about 35 per cent. of the endosperm nitrogen has passed into the growing embryos during 9–10 days' germination on the malting-floors. It has previously been shown that only about 10 per cent. of the nitrogen in an ungerminated barley of average nitrogen content, is in a form which will remain permanently soluble in water, so that on the assumption that the whole of this soluble nitrogen is also readily diffusible (capable of passing through the absorptive epithelium) and suitable for the immediate nutrition of the embryo when germination commences, it still falls short, by 25 per cent., of the amount of nitrogen which migrates into the embryo during 9–10 days' growth. Since the permanently soluble nitrogen of malt is about double that in the corresponding quantity of barley, it is evident that at least 35 per cent. of the insoluble proteins of the endosperm must be metabolised during the malting process and brought into a condition in which they can pass over into the embryo. These facts throw a considerable side-light on the questions involved in the chemical study of the nitrogenous constituents of malt, especially as regards the amino acids, amides, and peptone bodies, and the organic bases associated with them.

Culture of the Excised Embryos of Barley on Nutrient Solutions Containing Nitrogen in different Forms.¹—In connection with the absorption of nitrogenous substances by the barley embryo, the nutrient values of a number of nitrogen compounds were investigated by growing excised embryos in nutrient solutions containing these bodies. The nutrient solution contained, per 100 c.c., 0.100 gram of calcium sulphate, 0.025 gram of potassium chloride, 0.025 gram of magnesium sulphate, 0.025 gram of potassium dihydrogen phosphate, 5 grams of cane sugar, as well as two drops of N/5 ferric chloride solution per litre. The amount of nitrogenous substance added was varied so as to maintain a constant amount (0.042 per cent.) of nitrogen in the liquid, except in the case of tyrosine, which was too sparingly soluble. In this case the solution was kept saturated.

The barley embryos were cultivated for seven days, the increase in dry weight and nitrogen content then being determined. Thirty-five embryos were used in each experiment. In the control experiment (solution containing no nitrogen) the embryos increased during the culture, to the extent of about 2.7 times their initial dry weight, the absolute weight of nitrogen remaining constant and becoming, of course, less, relatively to the weight of the developed embryos. Both tyrosine and phenyl-alanine had a distinctly poisonous effect on the young plants. With phenyl-alanine only, there was a slight growth of the embryo, and in both cases the amount of nitrogen in the plantlets was materially reduced. Leucine also, at the above-mentioned concentration, must be regarded as directly tending to inhibit the growth of the plantlets; in this case, a very small absorption of nitrogen occurred, but the dry weight of the plantlets was less than that of those grown without nitrogen. The behaviour of the malt albumoses and malt peptones was much the same as that of leucine. In these cases, however, the retardation of growth, and small assimilation of nitrogen, may be due to the presence of substances other than the malt peptones and malt albumoses.

Choline, allantoin, and betaine all contributed nitrogen to the growing plantlet, and practically to the same extent. Choline and allantoin also increased the weight of the embryos; in the case of betaine, the weight of the embryos after development was the same as in the control experiment. Ammonium sulphate, aspartic acid, glutamic acid, potassium nitrate, and asparagine showed a progressive increase in their power to contribute nitrogen to the plant. Under the conditions of the experiment, asparagine was found to yield more nitrogen to the plant than any other nitrogenous substance hitherto examined. The nutritive value of aspartic acid is considerably less than that of asparagine (from which it is derived by hydrolysis). In this respect, asparagine and aspartic acid are analogous to cane sugar and its hydrolytic decomposition products (dextrose and levulose). Glutamic acid resembles aspartic acid in its nutrient value, and it is probable that the value of glutamine, the amide from which it is derived, would be found to approximate to that of asparagine.

¹ H. T. Brown, F. Escombe, A. McMullen, and J. H. Millar (*Trans. Guinness Research Laboratory*, 1906, 1, Part ii., 288-299).

A comparison of the results obtained with the nutrient solution to which asparagine had been added, with those obtained by allowing the embryos to germinate in contact with their natural endosperms showed that the amount of nitrogen assimilated from the endosperm during four days' culture was about the same as that absorbed from the artificial medium containing 0.2 per cent. of asparagine in seven days. When the asparagine was increased to from 1-2 per cent., about 50 per cent. more nitrogen was absorbed from the artificial medium in seven days than from the natural endosperm in four days. The dry weight of the embryos in artificial culture for seven days, however, never much exceeded that of the four days' germination under natural conditions, a fact which goes to show that, although the cane sugar was at optimal concentration, it was inferior to the nutritive power of the carbohydrate material in the endosperm.

The Water-Soluble Polysaccharides of Barley and Malt.¹ (Compare p. 477.)—In order to obtain the gums or "amylans" present in barley, the ground barley was treated with boiling water, liquefied at about 70° C. with malt extract, boiled again, cooled to 50° C., and saccharified. The solution was boiled and filtered, the filtrate and washings being concentrated to a sp. gr. of 1060. The syrup thus obtained was treated with three times its volume of 80-per-cent. alcohol (by volume). From the crude "amylans" which separated, the liquid was poured off after a short time, the "amylan" being washed with 60-70-per-cent. alcohol, reprecipitated with 80-per-cent. alcohol from water, washed with absolute alcohol, and filtered off. This product, the authors termed "crude amylan"; it generally possesses a copper reducing power of R 1.5-R 4, an ash content of 0.5-6 or 7 per cent., and a nitrogen content of 0.1-0.8 per cent. The crude amylan, on hydrolysis with oxalic acid, yielded about 10 per cent. of tartaric acid (racemic or meso-) which was derived from the C₅ complexes of the amylans. The remainder of the hydrolytic products (*i.e.*, after removal of the tartaric acid) was found to be fermentable by yeast to the extent of 60 per cent. The only fermentable sugar present in the hydrolytic product of the "amylans" appeared to be glucose. Of the non-fermentable part, about 20 per cent. appeared to consist of pentoses, the remainder being a substance, or substances, of low rotatory and reducing powers, the nature of which is at present unknown.

An investigation of the formation of "amylans" during the growth of the barley grain, appeared to show that the formation of "amylans" and starch represent two different sets of phenomena which are independent of each other, and that the amylans do not stand in any genetic connection to the starch.

The barley amylans, prepared as above described, were uniformly dextro-rotatory; by treating ground barley with boiling water, saccharifying with malt extract, and fermenting the liquor with yeast, a solution was obtained, from which a considerable amylan-like precipitate was thrown out with excess of alcohol. The precipitate was dissolved in hot water, filtered, the filtrate

¹ H. T. Brown, F. Escombe, A. McMullen, and J. H. Millar (*Trans. Guinness Research Laboratory*, 1906, 1, Part ii., 312-337).

made alkaline with caustic potash, and again precipitated with alcohol, the precipitate being dissolved in hydrochloric acid and reprecipitated with alcohol until free from acid. The substance thus obtained, had $[\alpha]_D = -100.34^\circ$ and R 6.0; it contained 1 per cent. of ash and an amount of nitrogen equivalent to 1.6 per cent. of protein. It became dextrorotatory on hydrolysis, and yielded arabinose by this treatment. It is shown to be identical with the "araban" obtained by Wroblewski from malt.

A crude amylan was also isolated from malt ($[\alpha]_D + 118^\circ$), which, on hydrolysis with oxalic acid, yields chiefly glucose, a strong odour of furfuraldehyde being, however, observed during hydrolysis. No galactose was found in the products of hydrolysis; hence, Lindet's claim that galactans are formed during the malting process, appears to be unfounded. Another sample of malt amylan was dissolved in potash, reprecipitated with alcohol, dissolved in hydrochloric acid, again precipitated with alcohol, and then precipitated from aqueous solution with alcohol until neutral. The specific rotation of the product was $[\alpha]_D - 17.3^\circ$; on hydrolysis with oxalic acid, the rotation was changed to $[\alpha]_D + 37.6^\circ$, whence it would appear that the preparation consisted, to some extent, of the araban obtained from barley amyloins and from malt by Wroblewski's process.

Direct Determination of Air in Barley.¹ (Compare p. 438).—The following was found to be the best method for determining the air in barley. A weighed quantity of the barley was placed in a tube of known capacity provided with taps at each end. The tube was connected, through drying tubes (phosphoric anhydride), with a Töpler pump, and the latter with a Lunge nitrometer filled with mercury. The volume of dry air in the empty tube for containing the barley, was determined both for the air of the laboratory (mean of six days) and for dry air. All the air volumes actually measured were increased in the proportion of these numbers.

The specific gravity of the barley was found by the toluene displacement method, the volume of the weighed quantity of barley being calculated therefrom; the difference between the volume of the tube, and that of the barley, gave the volume of air surrounding the latter. The difference between the volumes of air pumped off, and of the air surrounding the barley, represented the "air spaces" in the grain. In the case examined, the volume of "air spaces" was found to be 5.4 per cent. of the volume of the grain. From the specific volume of the barley and that of the air-free barley substance, the volume of air spaces was calculated to be 9.2 per cent. The cause of this difference was next investigated. The discrepancy appeared to be due, in part, to the air under the pales, consequently the experiments were continued with a skinless barley. The specific gravity of the degermed grain and of the whole corn after removing the air from the germ by placing the corn with toluene in a vacuum, were determined. Since toluene cannot penetrate the endosperm, the latter determination gave the real specific gravity of the endosperm

¹ H. T. Brown, F. Escombe, A. McMullen, and J. H. Millar (*Trans. Guinness Research Laboratory*, 1906, 1, Part ii., 338-345).

with the attached air-free germ. The specific gravities of the degermed grain, and of the grain with air-free germ attached, were very nearly the same (1.391 and 1.388); the mean value 1.390 was taken to represent the apparent specific gravity of the skinless barley. The volume of "air spaces" in the skinless grain was found to be 2.4 per cent. of the volume of the grain as compared with 3.1 per cent. calculated from the specific volumes. Hence the endosperm contained 0.7 per cent. of interspaces which were either vacuous or filled with air which could not escape when the barley was placed in a vacuum. On rendering the barley mealy by soaking and drying, it was found that the "air spaces," found by direct determination amounted to the same volume as before (2.4 per cent.), whereas the calculated "air spaces" had increased to 4.9 per cent. It is concluded from this result, that the interspaces formed during this artificial acquirement of mealiness, are vacuous. This opinion was confirmed by observations made during the soaking of the skinless barley.

On steeping in toluene, the mealy and steely corns may be distinguished by their apparent whiteness and transparency respectively. On soaking in water, the steely corns became white in about six hours, *i.e.*, as soon as the integument of the barley was softened, and before the starchy contents of the endosperm were rendered moist, the sample became mealy. The mealiness is produced by expansion of the contents of the endosperm, the expansion being caused by a lessening of the stress which no doubt exists in the endosperm of a steely barley, and which is probably exerted by the aleurone layer. Since the grain became mealy even under air-free water *in vacuo*, the spaces, formed in the expansion, must be vacuous. The expansion radiates from the ventral furrow, *i.e.*, where the aleurone layer is thinnest. It is pointed out that the ventral furrow, and the thinner integument in that part of the barley, must protect the grain from rupture, which would probably otherwise occur, owing to the variations of volume during the growth of the grain.

The Existence of a semi-permeable Membrane enclosing the Seeds of some of the Gramineæ.—An exceedingly interesting piece of work has been carried out recently by A. J. Brown.¹ During an investigation of the conditions governing the absorption of water by the ripe grain of certain cereals, some experiments with the grain of *Hordeum vulgare*, var. *coerulescens* (see p. 431), indicated that the embryo and endosperm of the grain are contained within a semi-permeable membrane. (Compare pp. 253-255.) This variety of barley is characterised by the greenish-blue colour of the skin, which becomes particularly apparent after the grain has been steeped. This colour is due to a pigment secreted by the aleurone cells; it is changed to red by acids. In the course of some experiments in which the grain was steeped in dilute 1 per cent. sulphuric acid, Brown observed that only in those corns which were punctured or otherwise damaged the blue colour changed to red. The undamaged corns, on the other hand, remained

¹ *Annals of Botany*, 1907, 21, 79.

blue, indicating that the acid had not obtained access to the interior of them. It was further noticed that this condition remained for a week or more, if germination were prevented or delayed by keeping the grain submerged in the acid. As soon, however, as germination took place with consequent rupture by the growing roots of the skins of the grain where they protect the base of the embryo, the blue colour speedily changed to red. It would appear, therefore, that when this variety of barley is steeped in 1 per cent. sulphuric acid water alone enters the grain. This was proved to be the case as follows: A known weight of the barley was steeped in a known volume of standard normal sulphuric acid, the steeped grains were then re-weighed and from the increase in weight the amount of imbibed water was calculated. After this the acid content of the residual liquid was determined by titration, when it was found that a concentration had taken place which was proportional to the water imbibed by the grain. Evidently, then, the membrane which surrounds the embryo and endosperm is permeable to water but not to the acid. Subsequent experiments showed that the barley might be steeped in solutions of sulphuric acid of such high concentrations as 18 and 36 per cent. without the germinating power being impaired. After being steeped in solutions of this strength, if well washed with water and placed under favourable conditions, the corns germinated in a few days. The barley was steeped in 5 per cent. solutions of copper sulphate, ferrous sulphate, potassium chromate, silver nitrate, and potassium ferrocyanide, with the result that none of these salts was found within the coverings of the grain after three days' steeping, although in all cases the embryos and endosperms were distended and softened with water. In the case, however, of solutions of alkalis, such as 1-per cent. sodium hydroxide, the coverings of the grain were destroyed and ultimately disintegrated. Steeped in a 0.5-per-cent. solution of sodium hydroxide, however, the coverings of the grain remained intact and water alone entered. The membrane is not impermeable to iodine, and when steeped in an aqueous solution, the dissolved iodine as well as water gains access to the interior. Special experiments showed that the semi-permeable property of the covering of the grain is located in the spermoderm. The grain of *Avena*, *Triticum*, and *Secale* is also enclosed in a semi-permeable covering similar to that of the grain of *Hordeum*. The investigation is only in its preliminary stage, yet, so far as it has been carried, the results are of the greatest practical importance. If all varieties of barley contain the semi-permeable membrane it follows that the saline composition of steep water is only of importance in regard to those corns which are broken.

"**Bios,**" a new Substance indispensable to the Development of Yeast. (Compare page 337.)—In 1901 E. Wildiers¹ recalled the controversy between Pasteur and Liebig, in which the former stated that a quantity of yeast of the size of a pin's head was sufficient to set up vigorous fermentation in a solution containing sugar and mineral salts only, whilst Liebig failed to obtain this result. Pasteur's view has now been accepted generally, and

¹ *La Cellule*, 1901, 18, 313.

several purely mineral nutrient media, such as Pasteur's, Hayduck's, Naegeli's, and Raulin's solutions have come into vogue. Wildiers showed, however, that some other substance besides mineral matter and sugar was necessary for the development of yeast; and this substance he called "bios." He employed an approximately 10-per-cent. solution of sugar containing magnesium sulphate, potassium chloride, ammonium chloride, and di-sodium phosphate, of each 0.25 per cent., with 0.05 per cent. of chalk. In order to avoid introducing "bios" with the seed-yeast, the sowings had to be made with an extremely small quantity of yeast. When this was done, neither fermentation nor reproduction took place. Wildiers was able to determine, by gradually increasing the quantity of yeast sown, the minimum amount of yeast necessary to set up fermentation. This limit was found to correspond with the quantity mentioned by Pasteur. He also showed that the fermentation set up when the quantity of yeast sown was above the minimum limit, was not due to the increased number of living cells, but to the introduction of a chemical substance with the yeast. This was proved by the fact that the addition of increasing quantities of an emulsion of boiled yeast enabled minute quantities of living yeast (below the minimum) to exert their full activity. Further it was shown that a filtered decoction of yeast had the same effect.

The substance "bios" has not been isolated; it is soluble in water, but is not extracted by absolute alcohol or ether; it is, however, soluble in 80-per-cent. alcohol, hence Wildiers concluded that it is not lecithin. "Bios" is not present in the ash of yeast. It is not destroyed by boiling for half an hour with 10 per cent. by weight of sulphuric acid; it may, therefore, be a basic substance. It is probably destroyed by boiling for half an hour with 1 to 5 per cent. caustic soda. It is not precipitated by lead acetate, and the solutions are not affected by the removal of the lead with sulphuretted hydrogen. The solutions after removal of the lead give only slight precipitates with phosphotungstic or phosphomolybdic acid, basic lead acetate, silver or mercury salts, and the filtrates from these, after removing the metals, are all strongly active. Wildiers tested a large number of nitrogenous compounds which are not precipitable by metals, but failed to identify any of them as possessing the properties of "bios." "Bios" diffuses very easily through parchment paper; it is always found in Liebig's meat extract, in commercial peptones, and in beer-wort before sowing with yeast; it could not be detected in the products of digestion of pure albumins. During its multiplication and fermentative activity, yeast does not produce any fresh quantity of "bios." "Bios" is a substance which is probably still active at a dilution of about 1 part in 10,000. The experiments were carried out with a top-fermentation, variety of *S. cerevisiae* I, but other top-fermentation yeasts and a bottom-fermentation yeast yielded the same results.

The question of the existence of "bios" has been a subject of dispute ever since the hypothesis was put forward. Among those authorities who took up an adverse stand towards the question may be mentioned Fernbach, Krieger,

and Henry. On the other hand W. Windisch, who was one of the first to draw attention to the work of Wildiers, has preserved an open-minded attitude on the question. In 1903 Amand disposed of the view that "bios" is a substance which acts as an antidote towards poisons, and Kossowitz in the same year showed that "bios" is formed by bacteria, and that as soon as a mineral solution becomes bacterially infected, it is capable of serving as a nutrient medium for yeasts; in fact Kossowitz ascribes Pasteur's success in cultivating yeasts in mineral media, to a symbiosis of the saccharomycetes and the schizomycetes.

Quite recently R. Devloo¹ claimed to have isolated the active principle of "bios" in an approximately pure form, and he designated it by the name *biosine*. This is a mono-amino organic base, similar to, but not identical with choline. It occurs, together with about an equal proportion of choline, as one of the bases of ordinary lecithin. The amino group still contains one unsubstituted hydrogen atom, since the base is precipitated by a mixture of mercuric chloride and barium hydroxide, and is soluble in phosphomolybdic acid. Biosine is readily soluble in water, and is non-volatile; its hydrochloride, sulphate, and oxalate are soluble in water, and in 75 per cent. alcohol. It is incompletely precipitated by alcohol of above 80-per-cent. strength, and by mixtures of 80-per-cent. alcohol with ether, acetone or chloroform. Choline and its decomposition products have none of the physiological properties of biosine, which has not been identified with any of the known bases. Biosine is widely distributed in nature, and can be prepared from commercial lecithin, or more cheaply from dried yeast. According to quantitative measurements with purified biosine in mineral nutrient media, the base is completely assimilated by yeast.

The Fermenting Power of Yeast is determined by the Constitution of its Nitrogenous Food. (Compare page 344.)—H. Pringsheim² has observed that yeast only ferments sugar when it has at its disposal as source of nitrogen, compounds containing the residue —NH—CH—CO which residue,

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he points out, has a special significance inasmuch as, according to E. Fischer, the amino-acids of the proteins occur in amide-like chains of such residues. Further he shows that yeast can be grown in a 10-per-cent. solution of sucrose containing small amounts of potassium hydrogen phosphate, magnesium sulphate, traces of common salt and ferrous sulphate, and certain nitrogenous substances, without inducing alcoholic fermentation. These nitrogen compounds are sulphanilic acid, metanilic acid, naphthionic acid, aniline and its salts, benzamide, benzylamine, acetamide, acetanilide, methylaniline, diphenylamine, dimethylaniline hydrochloride, and pyridine. The addition of a compound containing the residue —NH—CH—CO—to such an inactive

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solution causes alcoholic fermentation to commence at once.

The Physiological Condition of the Cell and its Importance for the

¹ *La Cellule*, 1906, 23, 361.

² *Ber. d. Deutsch. chem. Ges.*, 1906, 39, 4048.

Technology of Fermentation Industries.—Professor M. Delbrück gave an interesting lecture on this subject before the London section of the Institute of Brewing last July.¹ He dealt with researches which have been proceeding for some years under his direction at the *Institut für Gärungs Gewerbe, Berlin*. He referred in the first place to the observation of the late M. Hayduck that the diastatic activity of malt was dependent on the amount of protein contained in the grain; and further to that of Wolf, who showed that the amount of heat produced by germinating barley in unit time was also dependent on the protein content. From these researches he had derived his conception of the heat-producing power of barley which was conditional upon the amount of protein present, the kind of protein, and the capacity of the grain to produce enzymes. The last-mentioned faculty was a racial quality, whereby the size of the grains was more especially a determining factor; for since the embryo of the grain was the real producer of enzymes (compare, however, p. 302), the greater the number of embryos and, therefore, grains present in a given weight of barley, the greater the amount of enzymes.

It was pointed out that the enzymes produced during the malting process worked together in a certain sense; starch was hydrolysed by diastase and the products were oxidised by the oxidase; peptase degraded the proteins, and thus provided nourishment for the plantlet, as well as raw material for the formation of enzymes generally. But in spite of enzymatic forces there occurred also, simultaneously, a reverse action—the reconstruction of starch from sugar; this had been observed especially during the drying of malt (*see* p. 465). This fact, and the observation that the protein degraded on the malting-floors was reconverted into protein during the drying process, justified drawing an analogy between the drying of malt and the ripening of grain on the stalk, as well as with the post-ripening phenomena which occurred subsequently to the harvest. Combining these observations with others concerning the synthetic effect of enzymes, Professor Delbrück drew the following picture:

“In the leaves, constructive enzymes are active. From simple compounds, starch and albumin are being built up, but at the same time the enzymes bring about a destructive or degrading change; they supply the grain with sugar and amides. In the ripening grain the constructive activity is predominant; during the malting process the degrading activity comes to the fore, but during the drying process the building-up or constructive metabolism again gets the upper hand. It must be recognised, therefore, that the physiological condition of the cell differs according to which of these two tendencies—the constructive or the degrading—is predominant. Which of the two tendencies shall gain the ascendancy is again dependent upon temperature, upon the amount of water present, upon aëration, and the various necessities for the life of the organisms.”

The degrading process was here compared with auto-digestion, but it was

¹ *Journ. Inst. Brewing*, 1906, 12, 642.

pointed out that the constructive activity during the ripening brought about a state of quiescence, and that there was also to be observed a reduction in the amount of enzymes during the ripening or drying process.

The purpose for which any particular grain was intended must determine the manner of its cultivation and the treatment it should undergo; thus, the distiller required a malt rich in diastase, one might go further and say, a malt rich in enzymes. For this purpose a small-grained corn rich in protein must be chosen. But the brewer's requirement was the converse. He required a "gentle" grain, and the quieter the degrading process went on during the malting the better; the malting loss was less, the sprouting not so conspicuous, and from a "gentle" barley, poor in protein, a larger amount of clean brewing malt and one which was richer in potential extract was obtained.

F. J. Hoffmann had shown that all these conditions were of importance in determining the baking quality of flour, and he had, therefore, agitated for the introduction of a method of buying and selling cereals according to their dry substance. These remarks were equally applicable to the judging of hops which could be stored for a considerable period.

The lecturer then observed that such plants as the potato and the beet, were, to a high degree, subject to chemical or physical changes both during growth and after they were gathered, this being due to the large amount of water they contained. Invert sugar was converted into starch in the case of potatoes and into cane-sugar in the case of the beet. To how great a degree these enzymatic activities were dependent upon exterior circumstances had been fully demonstrated by Müller-Thurgau, who had shown that a lowering of the temperature of the potato was accompanied by the formation of sugar from starch, and that a rise in temperature caused a reverse activity—starch being then produced from sugar. A ripe potato contained nearly the whole of its starch in the form of starch-meal; an unripe potato showed a greater proportion of sugar, but this percentage of sugar varied also, according to the race, the kind of manure used, and the climatic conditions prevailing during growth and at the harvest time. It had been proved by W. Henneberg, that sugar-containing potatoes were apt to rot and react with intensity upon the inoculation of the rot-producing bacteria. This confirmed the lecturer's view that the physiological condition of the potato cell, its capacity to form sugar, *i.e.*, its enzymatic capacity, afforded a standard by which its power of resisting the rot-producing bacteria could be gauged. His conception of the matter was thus stated: "The rotting of the potato is due only in the second place to the bacteria, and the way to the formation of these bacteria is already existent in the tendency of the potato itself towards destructive or reducing metabolism. An exceptional rôle is played in this process by the oxidase, which, inasmuch as it produces warmth, paves the way for the initial internal changes, which, in turn, are aided by the bacterial activities. The task which now faces the potato cultivator is that of choosing and raising a kind of potato which may be described as gentle, *i.e.*, a potato in which the constructive tendency is predominant. The importance of the chemical com-

position, the tendency towards change, may easily be demonstrated in a simple experimental manner. If 1 kilo of potatoes be sliced and placed in a vessel protected against loss of warmth, but allowing access of air, the potatoes soon undergo a self-warming process which within a period of 20 hours produces an increase of temperature of from 10–20° C. It seems that there is a combined micro-organic and enzymatic influence at work. Certain kinds or ‘races’ of potatoes may be said to be ‘cold’ potatoes, others again may be called ‘energetic’ or ‘warm.’ A similar experiment may be made with barley. Barley roughly ground, moistened with a little water, and placed in vessels, similar to those used for the potatoes, will increase in temperature 20° C. in the course of 20 hours. But here also the degree of [rise in] temperature depends upon the sort of barley chosen for the experiment, and upon the physiological condition of the cells.”

Passing now to a consideration of the physiological condition of the yeast cell the lecturer stated that it was at times doubtful which was the more important: the choice of a certain race of yeast, or the treatment of a suitable yeast in such a manner as to compel it to produce the desired characteristics which would fit it for certain industrial ends. They were in a position at present to state that it was necessary to single out a certain race of yeast. But this of itself was not sufficient, and the chosen species would continue to preserve its desirable peculiarities only so long as it was maintained in a certain physiological condition by means of suitable manipulation. The means towards attaining this end were: nutrition, aëration, mechanical and dynamical conditions (movement), quantity of pitching yeast used, the arrest or removal of the products of fermentation (alcohol and carbon dioxide), the addition of stimulants, &c.

Hayduck again had laid the foundation of these researches by showing that the chief characteristic quality of yeast, the power to excite fermentation, was dependent upon the amount of protein present. The greater the protein content the greater the fermentative power. But this quality developed in too high a degree was apt to produce inconvenient secondary phenomena. Hayduck had regenerated the yeast, reducing the protein content by cultivation in media free from nitrogen, at the same time promoting multiplication by an increased access of air. An early result of this had been the discovery of the law formulated by the lecturer, that the fermenting power of the yeast produced was in inverse ratio to the multiplication of the yeast-cells. Moderate multiplication produced a yeast rich in protein; a rapid multiplication, on the other hand, produced a yeast poor in protein. Everything that hindered multiplication, such as a low temperature, the shutting off of air, lack of movement, fermentation under carbon-dioxide pressure, conduced to the yielding of a yeast which was rich in protein and in fermenting power. On the other hand, a small quantity of pitching yeast, increased access of air, unrestricted movement, the removal of carbonic acid, produced a large quantity of yeast with very moderate protein contents and less fermenting power. If it were desired to obtain a rapid multiplication, combined with

high fermenting power, it was necessary to add a quantity of easily assimilated nitrogenous matter to the nutritive solution. This was done in the modern aërated yeast factories, malt combs being used as mash material.

Since Buchner's discovery of zymase, Hayduck's law, which stated that the fermenting force depended on the protein content, had now to be supplemented by the further condition that it depended upon the conversion of this protein into zymase.

H. Lange had established the fact that the liquid obtained by submitting yeast-cells to pressure could only possess a high fermentative action when it was obtained from a yeast rich in protein. Further, it had been ascertained that it was possible to alter the freshly-pressed yeast in respect to its content of zymase, by a suitable manipulation. In yeast, kept at a low temperature, the amount of zymase increased; in yeast kept at a higher temperature it decreased. Each species or "race" of yeast required a certain storage temperature, which must not be exceeded if the fermentative strength was to be maintained. The "cold" yeasts (produced by sedimentary fermentation) were exceedingly sensitive; the "warm" or surface-fermentation yeasts could support unharmed somewhat higher temperatures. But this falling-off in the fermentative power was at the same time closely associated with a development of the proteolytic powers in the yeast which at first induced a softening, and immediately afterwards led to a complete liquefaction of the yeast. In a certain sense, therefore, zymase and the proteolytic enzyme were antipodes. But the latter also belonged to the reversible enzymes, it built up and broke down, according to the surrounding influences and to the physiological conditions. A constructive enzyme was present in yeast, since glycogen was formed from sugar and set apart as a reserve substance; fat globules were also formed from the nutritive material; further, amides were built up into protoplasm and reserved protein. The lecturer pictured an army of enzymes hurling itself against the entering nutritive mixture; each unit was at first actively destructive, in order immediately afterwards to begin its constructive work, either in providing the young cells with building material, or in setting apart reserve substances.

An unsuitable manipulation of the yeast produced a preponderance of proteolytic enzyme, the protoplasm underwent auto-digestion, and the enzyme, which was indispensable for the preparation of the nutritive materials for the yeast, and for the building-up of protein, became, under these circumstances, a deadly enzyme. Over modification in the malting brought about the auto-digestion of the malt. The regulation of the activity of the enzymes was one of the necessary conditions of life. It should be the aim, always and everywhere, to strive to attain a certain physiological condition, to serve particular technical ends corresponding to the physiological functions of the organs. A preponderating proteolytic activity occurred as soon as the optimum temperature was in the slightest degree exceeded. An unsuitable temperature thus caused the yeast-cell to become diseased, and this might be described as an enzymatic disease. But yeast

could bear a temperature, which was in itself injurious to it, if it were introduced into a nutritive solution containing sugar; here the yeast was stimulated to renewed activity, and its vital energy and fermenting power were increased.

H. Lange's latest experiments had been carried out with a view of establishing the effect of certain bodies on yeast. The introduction of minute quantities of acids, among which might be mentioned the fatty acids, the addition of phosphate of potash, which had in this case no nutritive value, and the employment of rare compounds (which had generally very little to do with the fermentation industry) such as, for example, urea, made it possible, under special experimental conditions, to increase the efficiency of the yeast three-fold. It had often been asked, what important physiological significance the peculiar dynamic effects of the splitting up of sugar into alcohol and carbon-dioxide might have. It was very easy to reply that a sort of subtle respiration process was going on here—that the cleavage was a hidden source of heat; but the significance of this activity was, particularly from a zymotechnological point of view, far more comprehensive. It was known that the most powerful defensive agencies of the yeast against the attacks of foreign organisms lay in its fermentation energy. The lecturer had always looked upon the fermentative effect of the yeast in this light, and had demonstrated that its organism, in sending out carbonic acid and alcohol, thus protected itself against all the organisms for which these substances were poisonous.

The effect of the carbonic acid was ten times as deadly as that of the alcohol. It was, for this reason, a universal preservative for all kinds of liquids as it made them unfitted for developing living organisms. The conclusion was thus arrived at that zymase was not only a respiration enzyme, but also a fighting enzyme; and that the stimulating action, which could be brought to bear on the yeast, and which resulted in the production of zymase, signified that the yeast was putting itself into an attitude of defence. Once this conception was grasped, a chain of reasoning was set up which, of itself, led to the discussion of the question whether there were not also other enzymes which form a part of the fighting organisation of yeast. And here the proteolytic enzyme stepped once more into the forefront, for it was the death-dealing enzyme, inasmuch as it immediately attacked all inimical organisms, dissolving and killing them.

Coagulase, which reduced the foe to a harmless and quiescent state by a process of imprisonment or envelopment, might be looked upon in a similar manner.

That the proteolytic enzyme played a decisive part in preserving the yeast in a healthy condition was confirmed by the interesting observations of Leonor Michaelis, who had pointed out that foreign albuminoid matter was, under all circumstances, a deadly poison for the organism, and that this was rendered innocuous by the proteolytic enzyme which degraded it. In the struggle for life, those organisms which specialised in the production of fighting substances and in the cultivation of fighting enzymes, would be the

strongest. This was true not only as regards plant life, but also with respect to animal organisms as well. Especially applicable was this conception with regard to the micro-organisms. Lactic acid bacteria possessed means of defence in the lactic acid enzyme as did the butyric acid bacteria which produced butyric acid, a substance which was pernicious in its effects on other organisms. During the course of these researches, Lange succeeded in observing a phenomenon which was calculated to excite the greatest possible astonishment. Bruised grain (or bran) and meal had always been regarded, the whole world over, as amongst the most harmless stuffs, equally valuable as a nutritive material not only for mankind and animals, but also for all kinds of fungoid growths and bacteria. Of course, for certain kinds of fungi, slight alterations as, for instance, a process of saccharification were undergone, but nobody had concluded from this fact that it might, in poorly saccharified mashings, exercise a distinctly poisonous effect, and yet this was the case. It was universally true that in varying degrees in certain concentrations applied to certain quantities of yeast, bruised grain or meal, or even a watery extract of these, had such a poisonous effect on yeast, that after the lapse of a few minutes even as many as 90 per cent. of the living cells were killed! Naturally this was the case only when the yeast possessed a certain degree of sensitiveness, or rather susceptibility; the distiller's yeast races (the top fermentation type of yeast) were capable of offering resistance, but this resistive power was less marked in the brewer's top fermentation yeast, and the most susceptible of all was the bottom fermentation type of brewer's yeast.

The question arose were these pernicious substances alkaloids, or might they not also turn out to be fighting enzymes, antitoxins, which were met with everywhere in nature? Or were they albuminoid forms? There was some probability that the phenomenon in question belonged to the albuminoids and to the enzymes produced by them; for the poisonous effect could be neutralised by heating the grain or the watery extract of grain.

The different kinds of grain varied also in this respect to a considerable degree; corn and oats did not possess this poisonous quality, wheat had it to a certain extent, but the most poisonous of all were barley and rye.

The malting process minimised the poisonous effect, or did away with it altogether. It was thus seen that it was those cereals which had the greatest durability, and which were as it were, best equipped with "keeping" qualities, which possessed means of defence; and, indeed, they were very necessary for them. Life in and of itself was healthy, for it fought with the living enzymes, but the quiescent or resting organism was exposed to any and every assault, and it needed for this reason special means of protection. It must now be recognised that the vegetable organisms had also their weapons in the struggle for life. Formerly these weapons had almost exclusively been observed in the world of micro-organisms.

The lecturer submitted that if this train of thought were traced to its logical conclusion, the plants must be followed "from the cradle to the grave." In all cases it must be sought to establish the existence of such a

fighting force as he had alluded to, and the science of plant-rearing, which had hitherto contented itself with a recognition of the facts that certain sorts of corn readily succumbed to the attacks of fungi, that certain kinds of potatoes and of beets fell easy victims to rot, must from this day forth, in each individual case set itself the question: Was this weakness in any way caused through a lack of effective fighting power?

Appended to the lecture are the following examples:

400 c.c. 10 per cent. cane sugar solution (distilled water), 10 gr. yeast, carbonic acid determined by volume.

I. Stimulation with Ground Oats.

—	Beer yeast without additions.	Yeast with 5 gr. ground oats.
After 1st half-hour . .	c.c. CO ₂ . 60	c.c. CO ₂ . 85
" 2nd " . .	156	378
" 3rd " . .	180	430
" 4th " . .	212	384
Total . .	608	1277

*II. Checking or Retarding Effect of Ground Rye in Quantities of
from 5-20 grs.*

—	Beer yeast only.	+ 5 gr. ground rye.	+ 10 gr. ground rye.	+ 20 gr. ground rye.
After 1st half-hour . .	c.c. 38	c.c. 10	c.c. 0	c.c. 18
" 2nd " . .	250	8	14	8
" 3rd " . .	386	10	10	8
" 4th " . .	420	20	10	8
Total . .	1094	48	34	42

III. Checking with Peptone Rich in Albumoses.¹

—	Beer yeast alone.	The same yeast + 1 gr. added peptone.	5 gr. peptone.
After 1st half-hour . .	c.c. 56	c.c. 8.	c.c. 26
" 2nd " . .	120	28	86
" 3rd " . .	204	62	140
" 4th " . .	240	86	186
Total . .	620	184	438

¹ The checking influence is paralysed by addition of greater quantities of peptone probably caused by better nutrition of the yeast.

IV. *Stimulation with Peptone Poor in Albumoses.*

—	Beer yeast alone.	+ 1 gr. peptone.	+ 5 gr. peptone.
	c.c.	c.c.	c.c.
After 1st half-hour .	56	19	18
„ 2nd „ .	116	120	220
„ 3rd „ .	198	280	448
„ 4th „ .	250	354	470
Total .	620	773	1156

V. *Checking Action of the White of Egg.*

—	Yeast alone.	+ 5 c.c. white of egg.
	c.c.	c.c.
After 1st half-hour . .	52	40
„ 2nd „ . .	196	80
„ 3rd „ . .	266	106
„ 4th „ . .	296	124
Total . .	810	350

Summary of the Results obtained in the Experiments Relating to the Toxic Action of Cereals on Low-fermentation Beer Yeast.¹ (Collaborators: Dr. H. Lange, Dr. Henneberg, Dr. F. Hayduck, Dr. Wendel.)—In the *Jahrbücher* of the Versuchs-und Lehranstalt für Brauerei, for 1904 and 1905, it was mentioned that a large number of inorganic and organic substances have a stimulating effect on the fermentative activity of top-fermentation yeasts, especially distillery yeasts.

The study of these stimulants having been extended to bottom-fermentation beer yeasts, the remarkable result was obtained, that certain cereals, far from having a stimulating effect, as in the case of distillery yeasts, exhibited an extremely toxic effect. This effect was determined, both by the Hayduck method of measuring the activity of yeast, and also microscopically.²

The toxic action only becomes manifest when the yeast and cereals are present together in saccharine solutions prepared with *distilled* water.

From the abundance of experimental material at hand, the following points may, up to the present, be regarded as proved:—

1. Rye, wheat, and barley, in the form of grits or flour, placed with bottom-fermentation beer yeast in a solution of saccharose, will kill up to 99 per cent. of the yeast in a few minutes. When this was followed by the Hayduck method the action was manifested by an almost complete absence of fermentation. Maize and oats do not show this toxic action.

¹ *Journ. Inst. Brewing*, 1906, 12, 666.

² In most instances the two tests gave identical results.

2. By agitation in distilled water, the flour of rye and wheat furnish extracts that are also toxic toward beer yeast, but to a far less extent than the corresponding solid substances themselves. More powerful extracts are obtained with diluted glycerin. Extracts nearly as strong in action as the corresponding cereal can be obtained by stirring up wheaten flour in distilled water and treating it with ground yeast, or better, pepsin.

3. The poisonous substance is confined to the grains of rye, being absent from the chaff, husk, stalk, roots and leaves of the plant.

4. The following points are important in the characterisation of the poison :—

(a) The protein sludge separating from the coarser particles when rye grits are shaken up with water, is specially poisonous.

(b) The same effect is manifested by the glutinous mass obtained by kneading wheaten flour under water.

(c) The toxic action can be destroyed by heating rye grits for several hours at 100° C.

(d) The same effect is produced by boiling the poisonous extract. (In some cases, however, the toxic action was merely weakened, not destroyed.)

(e) Malting the cereal weakens the toxic property, but whether it can entirely destroy it cannot be determined from the conflicting results obtained.

(f) No poisonous substance can be extracted from rye grits by ether and alcohol, and the same effect is produced by the grits after the extraction as before.

(g) No poisonous substance can be isolated by steam distillation, whether in an acid or alkaline solution.

(h) No alkaloids could be detected.

From the experiments referred to under *a-e*, it seems probable that the toxic substance must be sought among the proteins, or may be produced from the same by the activity of the yeast.

5. All these toxic effects are completely obviated by the addition of a small quantity of inorganic salts to the solutions, lime salts being the most effective, and next to them magnesia salts. A partial or complete removal of the toxic action can be effected even by replacing distilled water by tap water, the effect of the latter being in proportion to its lime or magnesia content. This remarkable fact makes it seem probable that the poisonous substance enters into combination with the aforesaid salts or their metals. Should the matter be a case of the fixation and precipitation of an acid, many points seem to indicate oxalic acid, since this acid, even in a concentration of 0.05 per cent., will completely kill low-fermentation beer yeast in solutions of saccharose in distilled water, and it is also well known to occur, as a lime salt, in yeast and beer.

6. Other substances exerting a strongly poisonous action on low fermentation beer yeast are : egg albumin, Witte's peptone (but not Aschmann's), and papayotin (a tryptic enzyme from melons).

7. The resisting power of low-fermentation beer yeasts toward the cereal poison is also dependent on their physiological condition.

8. Wheaten flour seems also to exert a toxic action on the following micro-organisms; but this requires confirmation :—

- (a) High-fermentation distillery yeast (after warm storage).
- (b) Film yeasts and torula.
- (c) *Mucor Rouxii*, *Aspergillus oryzae*, *Oidium*, *Penicillium glaucum*.

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